

**The interactive effect of pain and topical analgesic on motor pathway
excitability of the biceps brachii.**

By

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ABSTRACT

Delayed onset muscle soreness (DOMS) is muscle pain that arises 24-72 hours after exercise involving unfamiliar strenuous muscle loading. DOMS is commonly induced by eccentric muscle contractions and may signify exercise-induced muscle damage including impaired excitation-contraction coupling and injury to cytoskeletal proteins in the sarcomere. During DOMS, muscle pain is transmitted via A-delta and C-fibre afferents (group III/IV afferents) to the central nervous system (CNS). While it has been shown that muscle force production is significantly diminished in DOMS, there is limited evidence on the influence of DOMS on corticospinal excitability or inhibition. Furthermore, to date no work has investigated how the application of topical analgesic interacts with DOMS to modulate corticospinal excitation. The current thesis employed a model of DOMS involving fatiguing eccentric elbow flexor contraction exercise. We investigated the effect of DOMS on indices of corticospinal excitability (motor evoked potential [MEP] and cervicomedullary evoked potential [CMEP] area and latency) and inhibition (silent period), peripheral motor excitability (Mmax area and latency), and pain pressure threshold (PPT) in the dominant biceps brachii muscle, as well as how application of a menthol-based topical analgesic (Biofreeze®) influenced these outcomes. Two experiments were employed to test the effects of topical analgesic in participants with and without DOMS. In the first experiment (*Experiment A: No DOMS*), 16 young healthy adults (F = 8, M = 8; age = 23 ± 1.1 yr; body mass = 71.9 ± 9.1 kg; height = 174.2 ± 8.2 cm) were randomly allocated to receive either a menthol-based topical analgesic gel (Topical Analgesic, $n = 8$) or a placebo gel (Placebo, $n = 8$) in a double-blind fashion, during a single session; DOMS was not induced. Prior to the application of gel (pre-gel), as well as 5 (post-5 min), 15 (post-15 min), 30 (post-30 min), and 45 (post-45 min) minutes after the application of gel, MEP area, latency, and silent period; CMEP area and latency; and Mmax area

and latency were measured. Participants' MEP and CMEP areas were normalized to Mmax area. Neither group showed a statistically significant change in these outcome measures at any post-gel time-point compared to pre-gel ($p > .05$). In the second experiment (*Experiment B: DOMS*), 16 young healthy male adults (age = 26 ± 5.1 yr; body mass = 81.9 ± 9.1 kg; height = 179.8 ± 6.1 cm) completed two experimental sessions. During the first session participants completed a fatiguing eccentric elbow flexor contraction protocol to induce DOMS. Participants returned 48 hours later and were randomly allocated to Topical Analgesic ($n = 8$) or Placebo ($n = 8$) in a double-blind fashion. During the second session MEP area/Mmax area, MEP latency, silent period, CMEP area/Mmax Area, CMEP latency, Mmax area, and Mmax latency were measured at the same time-points as above. Additionally, PPT was measured during session one (pre-DOMS), as well as at the above time-points. Both groups exhibited a statistically significant decrease in PPT from pre-DOMS to pre-gel (Topical Analgesic, pre-DOMS = 7.03 ± 2.48 kg, pre-gel = 3.12 ± 1.26 kg, $p < 0.001$ Placebo, pre-DOMS = 5.77 ± 2.35 kg, pre-gel = 3.51 ± 1.58 kg, $p = .005$). Following the application of gel there was a significant increase in PPT at post-15 min (3.70 ± 1.69 kg, $t_{(7)} = -2.619$, $p = .034$), post-30 min (3.92 ± 1.67 kg, $t_{(7)} = -3.987$, $p = .005$), and post-45 min (4.33 ± 1.65 kg, $t_{(7)} = -4.566$, $p = .003$) compared to pre-gel (3.12 ± 1.26 kg) in the Topical Analgesic group only. Under the Placebo group, there was no statistically significant change in PPT values ($p > .05$). Regarding neurophysiological changes there was a statistically significant increase in silent period compared to pre-gel (85.67 ± 19.65 ms) at both post-30 min (96.08 ± 26.62 ms, $p = .045$) and post-45 min (94.23 ± 22.32 ms, $p = .029$), in the Topical analgesic Group. The Placebo group did not exhibit a statistically significant change in silent period at any time-point ($p > .05$). No other measures of corticospinal (MEP area/Mmax area, MEP latency, CMEP area/Mmax area,

CMEP latency) or peripheral motor excitability (Mmax area, Mmax latency) were significantly different after the application of topical analgesic or placebo gel ($p > .05$).

When comparing pre-gel neurophysiological outcomes in participants from *Experiment A*: No DOMS ($n = 16$) and *Experiment B*: DOMS ($n = 16$), there were statistically significant differences in MEP area/Mmax area (*Experiment A* = 0.222 ± 0.169 ; *Experiment B* = 0.097 ± 0.057 , $p = .011$), CMEP area/Mmax area (*Experiment A* = 0.186 ± 0.148 ; *Experiment B* = 0.077 ± 0.045 ; $p = .012$), and silent period (*Experiment A* = 77.41 ± 31.05 ms; *Experiment B* = 100.85 ± 32.29 ms; $p = .045$). No other neurophysiological measures were significantly different across experiments ($p > .05$). The present findings suggest that DOMS imposes changes in the neuromuscular system that result in increased pain, reduced corticospinal excitability, and elevated corticospinal inhibition. Following the administration of menthol-based topical analgesic, but not a placebo gel, there is a reduction in pain which is accompanied by further increases in corticospinal inhibition. These results provide novel information on the neurophysiological effects of DOMS, as well as the influence on topical analgesic on DOMS-induced neurophysiological changes.

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LIST OF ABBREVIATIONS (in order they appear)

DOMS	Delayed onset muscle soreness
MEP	Motor-evoked potential
CMEP	Cervicomedullary-evoked potential
Mmax	Maximal muscle compound potential
MVC	Maximum voluntary contraction
PPT	Pain Pressure Threshold
TMES	Transmastoid electrical stimulation
TMS	Transcranial magnetic stimulation
CSE	Corticospinal excitability
ISI	Interstimulus Interval
CSP	Cortical Silent Period
SAI	Short afferent inhibition
EMG	Electromyography

CHAPTER 1 – REVIEW OF LITERATURE

1.1 Introduction

This literature review gives a brief overview of the current methods used to evoke and assess pain, the ways that pain is measured and the procedures used to assess corticospinal excitability. There is an interaction between sensory inputs and motor outputs from the cortex and spinal cord. These interactions are complex and while not completely understood they will be reviewed along with some of the methods used to assess these complex interactions. Also, techniques to attenuate painful input and the potential effect on corticospinal output will be highlighted. Finally, the physiology behind the effect of the topical analgesic menthol will be explored along with its effects on pain and motor output. Hopefully by understanding the flaws and limitations in the current methodologies as well as what's known about the etiology of pain and analgesics on the nervous system, it will allow us to produce better methods for testing and assessing pain and its influence on the corticospinal tract leading and subsequent motor output.

1.2 Physiology of Pain

Pain is a complex process initiated by stimulation of peripheral nociceptive receptors and summated as conscious perception in the cerebral cortex. The sensation of pain in skeletal muscle is transmitted by large thinly myelinated group III (A-delta fibre and small unmyelinated group IV (C-fibre) afferents. Both of these sensory neurons terminate in free or unencapsulated nerve endings predominantly found in the interstitial fluid and connective tissue between muscle fibres, with their cell bodies in the dorsal root ganglia. The A-delta fibres, believed to transmit sharp, prickling, stabbing type pain, are predominantly activated by mechanical deformation of tissue. The C-fibres, which carry dull cramping pain are predominantly activated by a noxious chemical environment [Marchettini, Simone [1]]. Chemical substances that elicit action potentials in these

fibres include bradykinin, serotonin, histamine, prostaglandin and potassium. These are released by various sources including white blood cells, macrophages and platelets, which can be stimulated by skeletal muscle activity [2]. The type III and IV afferents synapse with neurons in the dorsal horn of the spinal cord. Wide dynamic range neurons are located in laminae five and respond to many types of stimuli, whereas nociceptive specific neurons located more superficially in lamina one and two, respond preferentially to noxious stimuli. The major neurotransmitter for injurious input in the spinal cord is glutamate but many other molecules can stimulate nociceptive neurons. From the dorsal horn these nociceptive signals are transmitted via a number of ascending tracts, which first synapse to pain relevant brainstem areas involved in emotion, cardiorespiratory regulation, sensory perception and movement. The impulses are then relayed too multiple subcortical and cortical regions involved in integration of the sensory, emotional and cognitive aspects of pain, confirming the multidimensional landscape of the human pain experience. For a further comprehensive review see Millan et al. [3].

1.3 Experimental Pain

The assessment of pain is a major challenge in both laboratory and clinical settings. It is difficult to evaluate the effect of pain on human performance due to the complex nature of its interactions with the nervous system. Furthermore, the mechanisms underlying pain are poorly understood. To help improve this lack of understanding, human experimental pain models have been developed to provide a way to explore the physiological effects of pain and its effect on human performance under controlled settings. The mechanisms of induced experimental pain can be broken down into three different categories based on the area of stimuli, including pain evoked in the skin, muscles and visceral areas. Pain models that originate around the skin are induced by

thermal, electrical, mechanical and chemical stimuli, and all have been well developed due to the easy accessibility of the skin [4].

1.3.1 Thermal stimulation

The use of cold thermal stimulation through the immersion of an extremity in ice saturated water for one to two minutes at a temperature between 0-2 degrees Celsius has been found to activate nociceptive afferents [4]. The nociceptive input is proposed to originate in cutaneous veins and has been termed cold pressure pain that has been shown to activate both A-delta and C fibres [4]. Limitations to studying this type of stimulation have mostly been demonstrated in the assessment of vascular reacting analgesics. The testing of these drugs have produced conflicting results because any vasodilation or constriction would affect the perception of pain mechanistically and not actually block the pain from being perceived [5]. This alteration in pain perception would affect the reliability of the results as any changes found may be due to differences between participants pain perception [6]. Another method commonly used to invoke thermal stimulation is contact heat, often applied from a Peltier thermode or heat foil. The rapid heating of the skin can also activate both A-delta and C fibres, with A-delta activated first followed shortly thereafter by C fibres [7]. One of the major limitations with the use of contact heat is concomitant activation of nociceptors and non-nociceptive low threshold sensory receptors. These receptors can be activated by mere contact of the thermode to the skin. By activating other afferent nerves, you potentially inhibit the influence of the pain afferents or at least make their effect undistinguishable from afferent information as a whole [8]. Radiant heat, however, has been the preferential method of inducing thermal stimulation. Radiant heat is most commonly used to cause experimental pain through laser pulses. These pulses cause a pricking pain in the skin. Again pain evoked by laser stimulation has been shown to be mediated through A-Delta and C-fibres [9]. A major advantage

to this technique is the fact that the stimulus does not require any contact with the skin, avoiding the problem associated with contact heat. However, reflectance absorption and transmission can vary from participant to participant based on skin pigmentation, so the required intensity to reach the threshold for pain can be highly variable [8, 9]. This could be somewhat remedied by finding participants with a similar skin tone, however, in turn you would be effecting external validity with this kind of participant selection bias [6].

1.3.2 Electrical Stimulation

A variety of electrical stimulation devices can be hooked up to electrodes and placed on the surface of the skin. In turn, a variation of different stimulation intensities and durations can be given to any area of the body. This allows researchers to have some selectivity on which afferent fibres are activated [10]. However, electrical stimulation in some cases may bypass the specific afferents associated with nociception and activate nerves directly, thus decreasing the external validity of the measure by poorly representing a clinical or practical setting [6, 11]. The act of stimulating the afferent nerves directly, although the most common, is not the only use of electrical stimulation to elicit experimental pain. Electrical stimulation can be applied directly into the muscle or the viscera. In order for the technique to be applied directly to the muscle the researchers require small needle electrodes with un-insulated tips [12]. Stimulation of the viscera with this method has the added drawback of being an even more invasive procedure. Stimulation of the viscera is often used for determining basic pain mechanisms and neurophysiological assessments [13, 14].

1.3.3 Chemical Stimulation

Capsaicin is one of the most commonly used methods of chemical stimulation. It is applied through intradermal injection or placement of topical capsaicin cream. The application is intended

to trigger primary and secondary hyperalgesia. The use of 100 microliters evokes a burst of pain that is short lived and is regionalized to injection or the topical sites. It has been speculated that mostly C-fibres are involved in the pain induced by capsaicin [15]. Limitations of this method are unclear and studies that examine this method have found differing results. For example when Wallace et al. [15] tested the effect desipramine, a clinically proven treatment of neuropathic pain, to block capsaicin induced pain, there were no beneficial effects. In contrast, when Dirks et al. [16] tested another neuropathic pain pharmaceutical agent, gabapentin, suppression of hyperalgesia following heat-capsaicin sensitization was found.

1.3.4 Muscle Pain Via Hypertonic Solution Injection

Another method of experimental chemical pain stimulation can be achieved by infusing algogenic substances into the muscles. This injection mimics the inflammation and effects on performance that are often associated with DOMS [17]. Injection of hypertonic saline has also become a widely accepted methodology due its ability to mimic the effect of DOMS. Hypertonic saline activates the nociceptive afferents within the muscle fibre and bypasses sharper and more distinct pain sensory fibres in this skin [18]. Injection of saline has a closely related etiology to musculoskeletal pain both in its effects on motor performance and subjective quality [19, 20]. C - afferent fibres are activated by these saline injections and are perceived as a dull deep pain. Reliability can be an issue when using this type of methodology for experimental pain as the interindividual differences are large [21].

1.3.5 Muscle Pain Via Delayed Onset Muscle Soreness

Muscle pain can also be induced through unfamiliar strenuous muscle loading exercise, arising 24-72 hours post exercise (i.e. DOMS). The response is most commonly brought about by eccentric muscle contractions and signifies that there has been muscle damage. Muscle pain

associated with DOMS is transmitted via A-delta and C afferent fibres and has a very high external validity, as this is one of the most common forms of pain experienced in everyday life. The major limitation that comes with the induction of muscle pain is the fact that not all participants will reliably get DOMS after exercise. Pain associated with DOMS will be described in more detail in Section 1.5.

1.4 Measurement of Pain

Some of the most common ways that pain is quantified is via verbal descriptor or visual analogue scales (VAS), and in the case of muscle pain, a technique termed pressure-pain threshold (PPT). The verbal descriptor scale is a technique in which the participants are presented with a number of words and are told to choose the one which best matches the pain they are feeling. The VAS has the participant rate pain on a scale of 1-10 [22]. Both these techniques have a drastic problem with participant reactivity [6], as the participant may rank the pain lower than actually experienced due to the presence of the researcher. Pain Pressure threshold is a measurement of tenderness over a specific area of the body. Using a pressure algometer, the researcher applies pressure to the place of interest of the participant, who then identifies when a perceived sensation of pressure changes into a sensation of pain. This method has been shown to produce accurate, valid and reproducible results [23-27]. However, the method can have improved reliability if an average of multiple trials is performed. This yields a better estimation of relative tenderness [28]. To further improve reliability, it has been suggested that the first trial measurements be discarded and the average of 5 trials (minimum) should be taken [29].

1.5 Delayed Onset Muscle Soreness

The concept of DOMS first originated in 1900 in an article ‘Ergographic studies in muscular fatigue and soreness’, by Theodore Hough [30] The main findings were the appearance

of muscle soreness eight hours to four days post ergograph (spring weighted) experiments, and that the soreness elevated to its highest point twelve or more hours post testing. Hough also found that if he ran the resistance protocol again when the soreness was present, the discomfort would dissipate within five to ten minutes of the experiment. Today DOMS is described as muscle pain and stiffness arising 24-72 hours after strenuous, weight-loaded exercise which is unfamiliar to the participant [31], with the “peak” of the soreness appearing around the 48 hour time point, based on indicators such as largest reduction in range of motion, increase in subjective pain measures and muscle volume [32, 33]. DOMS is considered to indicate muscle damage brought about by exercise, but differs from the pain associated with muscle fatigue that occurs during or immediately following exercise. The exact etiology of DOMS is not fully understood, however, almost all research surrounding the topic agrees that it is most commonly initiated by eccentric exercise, as shown in studies that have examined the association between muscle pain and isometric, eccentric and concentric contractions, as well as static activities [31, 34]. Eccentric exercise results in greater disruption to muscle tissue than concentric exercise as fewer motor units are required to generate equal muscle force. As a result, tension is created over a smaller area, which could cause mechanical disruption in the muscle fibres or connective tissue components [31]. However, it has not been proven that injury to muscle cells is the definite mechanism which generates DOMS. In fact, one histological study showed myofibril and cytoskeletal remodelling and not damage which occurred in DOMS affected muscles [35]. Since DOMS first appeared in the literature, many theoretical mechanisms have been proposed to explain its origin. These mechanisms include mechanical muscle damage, muscle spasm, cellular inflammation, lactic acid build-up, damage to connective tissue, enzyme efflux and myofibrillar and cytoskeletal remodeling theories. It is more probable, however, that DOMS arises from a combination of these mechanisms.

1.5.1 Increased Lactic Acid Accumulation

Lactic acid accumulation theory has been mostly discarded as one of the mechanisms that leads to DOMS. Increased lactic acid accumulation during or immediately following an intense bout of exercise can cause acute pain, but this mechanism cannot be confirmed as a significant contributing factor towards delayed soreness. For example, an experiment by Schwane et al. [36] showed that muscle lactic acid returns to pre-test measures within 60 minutes following exercise. Also, they found no correlations between blood lactate levels and muscle soreness over a 72-hour period after exercise

1.5.2 Muscle Spasms

The muscle spasm theory speculates that there may be hyperactivity within the resting muscle following bouts of exercise [37-39]. Hyperactivity results in tonic muscle spasms which compress local blood vessels, causing a decreased blood flow to the muscle and accumulation of waste products and enzymes within and around the muscle. This accumulation stimulates pain receptors at the site of muscle damage. The research behind this mechanism has been controversial, with no increase in EMG activity (which would represent a muscle spasm) in sore muscles found from a few studies [40, 41], while others have shown the opposite [42, 43]. Even if the EMG research results were consistent, no relationship has been found to correlate the magnitude of EMG muscle hyperactivity and muscle soreness [38]. Along with the lactic acid theory, the muscle spasm theory has largely been rejected due to the inconsistency in results.

1.5.3 Muscle Tissue Damage

The muscle damage theory was first proposed by Hough [44], attributing the sensation of DOMS to be due to the disruption of the contractile components of the muscle, particularly seen at the z-line following eccentric contractions [42, 45-48]. As this area has been seen to be the

weakest portion of the contractile unit of the muscles [48], disruption of the z-line would be the primary location of structural damage in the myofibril and sarcomere [49]. Type II fibres have weaker and narrower z-lines and eccentric exercise favors the activation of Type II fibres. Therefore, this mechanistic theory could explain why eccentric exercise leads to increased DOMS and is supported by muscle biopsy findings [50].

1.5.4 Connective tissue Damage

The connective tissue theory focuses mainly on connective tissue casing surrounding the muscle fibres, termed myofascia [49]. After exercise the majority of DOMS is experienced near the myotendinous junction, the distal portion of the muscle belly [51]. This area contains a large amount of connective tissue relative to muscle tissue. As stated previously it is clear that eccentric exercise results in greater likelihood of DOMS and eccentric movements primarily target type 2 muscle. Type II muscles fibres have weaker connective tissue than Type I fibres and therefore would be more susceptible to stretch induced connective tissue damage due to greater stress in eccentric exercise [37, 44, 52]. This damage would stimulate mechanoreceptors, leading to the sensation of pain [53]. Hydroxyproline (OHP) is a specific marker of connective tissue breakdown. 48 hours after exercise OHP levels are the highest [40], which correlates strongly with perception of muscles soreness post-exercise [49, 54].

1.5.5 Enzyme Efflux

During exercise, metabolites are released into the extracellular space due to increased fibre degradation [45]. Two common metabolites, calcium and creatine phosphokinase, have been found to build up during eccentric exercise and are well established as indicators of muscle damage. Calcium is found in the sarcoplasmic reticulum of the muscle cell and if muscle is broken down it will release calcium directly. Extracellular calcium is therefore thought to be a direct indicator of

muscle damage [37, 45]. The accumulation of metabolites such as calcium have been shown to activate proteases and phospholipidases, which assist in protein degradation. This could cause even weaker z-lines [55]. Due to the increased tissue breakdown and metabolite release it is likely that nerve endings may be sensitized through chemical stimulation which would increase pain sensation.

1.5.6 Inflammation

In order to deal with metabolite release and protein/cellular degradation that occurs during muscle damage, inflammatory cells migrate into the affected area. Also, the build-up of histamine, kinins, prostaglandins, and potassium [45] attracts monocytes and neutrophils to the damaged area [56]. All of this results in worsening the edema [57]. As a result of the ionic gradient change, fluid from inside the muscle cell will osmose to the extracellular space, resulting in increased osmotic pressure. This increase will stimulate type IV sensory neurons. Research has shown that with the increase of osmotic pressure, monocytes and macrophages secrete substances that increase the sensitivity of type III and type IV sensory neurons causing increased pain sensation [45, 57].

1.6 The Effect of DOMS on Human Performance

After a bout of intensive unaccustomed exercise, DOMS will occur over a period of 24-72 hours, peaking around the 48 hour time period and eventually dissipating within 5-7 days. The structural damage and soreness to both the muscles and connective tissue may result in altered muscle function and joint mechanics [58]. This damage can result in performance impairments over this time period. Neuromuscular impairments such as altered joint kinematics and functional impairments are prominent. A loss in muscular strength [59], force perception [60], joint angle perception, maximal voluntary contraction [59, 61-64], decrease in muscle activation and

increased EMG to force ratios can all be attributed to a loss in proprioceptive functioning in the days following exercise [65]. Although these functional impairments do correlate with the onset and progression of DOMS, it is important to note that they each go through their own time course. For example impairment of isometric muscular strength has been seen to peak around the 3-5 day range and take anywhere from 33 to 89 days to fully recover [66]. Functional impairments such as perception of force and joint position are well researched and believed to be initiated from peripheral receptors in Golgi tendon organs and muscle spindles [67-69]. In contrast, DOMS originates at the muscle belly. This represents a possible explanation for the differing time courses between function impairments and DOMS. For a more comprehensive review on effects of muscle damage on motor performance see Byrne et al. [70].

1.7 Topical Analgesic

Menthol is a naturally occurring ligand for transient receptor potential member 8 (TRMP8). In its normal state, TRPM8 permits the flow of ions, usually calcium or potassium, through cellular membranes where they are located. When a temperature decrease is detected below $26 \pm 2^{\circ}\text{C}$ [71], the TRPM8 effects the peripheral nerve endings that are cold specific and non-nociceptive (A delta fibres) by increasing concurrent flow resulting in cold perception [71, 72]. Menthol acts presynaptically where the TRPM8 channels congregate on somatic sensory neurons. While Menthol is a ligand for TRPM8, it is not specific to only the TRPM8 channel and will interact with other transient receptor protein (TRP) channels such as TRPA1 and TRPV3 [73]. These ion channels have been shown to induce different sensations in patients. They may elicit pain and inflammatory symptoms in some cases, while, in others, these proteins have been shown to contribute to pain analgesia [72, 74]. The analgesic effect of menthol may also be mediated by mechanisms independent of TRP channel proteins. Menthol can activate several pathways that all

contribute to reduced nociception. Menthol can activate GABA_A-receptors, which could result in inhibition of nociception through central mechanisms [75, 76]. Menthol also affects voltage-gated sodium channels which inactivates the ability of sensory neurons to induce action potentials [77]. It was also found that menthol inhibits neuronal voltage-dependent Ca²⁺ channels [78, 79], nicotinic acetyl choline receptors, and serotonin-gated ion channels, all of which have been linked to pain messaging [80, 81].

1.8 Menthol and Human Performance

Menthol is a commonly used topical analgesic in gels which are meant to reduce DOMS post exercise through mechanisms previously stated. However, does this perceived reduction in muscle pain lead to any alterations in human performance? Few studies have investigated this using menthol. Johar et al. [82] assessed the use of menthol applied to the bicep brachii of untrained individuals and found that menthol did significantly reduce DOMS compared to ice application. Also, tetanic force was higher in the menthol group compared to the ice group. However voluntary force (MVC) did not follow this trend and was not significantly different between groups. These results may be due to voluntary force requiring activation of higher order systems which have not been affected by the topical application of the menthol whereas tetanic force occurs at the muscle level where sensory afferent fibres may already have been inhibited by the analgesic allowing for a higher motor output. These results slightly differ from those of Topp et al. [83] as they saw an elevation in maximum voluntary wrist flexion and extension strength with the application of menthol compared to ice application to the lower arm. However, there was no difference between the menthol and placebo control group, suggesting that menthol had no effect on strength production and in fact ice reduced power in untrained individuals.

1.9 Corticospinal Tracts

Voluntary movement is determined and controlled by both the corticobulbar and corticospinal tracts [84]. Both tracts originate at the motor cortex, however corticospinal fibres innervate the majority of the body through spinal motoneurons which travel to the limbs and core muscles. This tract is the most important for voluntary body movements such as single and multi-jointed movements. In contrast, the role of corticobulbar fibre motor nuclei is focused on controlling facial muscles so it is less studied in human movement trials. Techniques have been developed to stimulate different portions of the corticospinal tract, allowing researchers to access changes in excitability throughout the tract and better understand the etiology of what has occurred. These techniques are essential as the corticospinal tract seems to be easily altered by many variables such as pain, fatigue, position and movement.

2.0 Stimulation Techniques to Assess Corticospinal Excitability

2.0.1 Transcranial Magnetic Stimulation (TMS)

TMS elicits motor evoked potentials (MEP) recorded with EMG of the muscle. TMS is elicited over the motor cortex in the area of the motor homunculus associated with the muscle of interest. A variety of coils can be used depending on the experimental setup and the question to be answered. Using a generic circular coil is less focal and activates a large portion of the motor homunculus, whereas, a double coil figure eight set-up provides more focal stimulation. Both of these coils have pros and cons. The generic circular coil is placed horizontally over the vertex with a wide spread area of activation so even if the placement of the coil is altered slightly, similar areas of the homunculus will be activated. This increases the reliability even in less trained researchers [85]. However, when testing for corticospinal excitability we assume that only the motor

homunculus is activated but with such an indirect form of stimulation, excitation occurs in other areas of the brain which has been proven to have an effect on corticospinal excitability [86]. This activation in other regions could bias the results, decreasing the validity and reliability. With the figure eight coil we see the exact opposite effect. Due to a precise stimulation region, measures must be taken so that the coil is positioned over the exact same position of the skull. To achieve this goal, researchers have derived a plethora of techniques. One example is to have the participant wear a swim cap with the area of stimulation marked off. However, even with this method there are limitations as there is no way to ensure the cap does not move or that it is placed on the head in the same position each day.

TMS activates corticospinal neurones leading to the activation of motoneurones. By using epidural or single motor unit recordings, Lazzaro et al. [87] and Amassian et al. [88] have shown that there are multiple components of the MEP. Short latency direct waves (D-waves) which are direct activation of these motoneurones by a single synapse, followed by longer latency indirect waves (I-waves) which require multiple synapses to reach the motoneurone. High intensity TMS is best used for eliciting a D wave and is caused by direct depolarization of the initial axon segment of the corticospinal neurone. I-waves will occur 1.5 ms after a D wave, showing evidence of synaptic discharge. I-waves are elicited along the tract when TMS intensity is put above motor threshold [89]. These elicited waves can be altered by many different factors such as: modulation of neurotransmission (i.e., acetylcholine, norepinephrine, and dopamine) [90], neurotransmitters (i.e., glutamate, GABA), interneurones contacted by corticospinal tract cells and activity-dependent changes (i.e. voluntary contraction) [91]. MEP amplitude is affected by all these factors. However, MEP amplitude is not a result of cortical changes alone but changes at the spinal level

as well. Therefore, it can be hard to determine where the change in the corticospinal tract has occurred. Any alteration in MEP amplitude represents a change in the neuromuscular system [92].

Due to this ambiguity, it is important to be able to activate the corticospinal tract at a subcortical level which would allow for a better interpretation of responses evoked at the cortex [93-97].

2.0.2 Transmastoid electrical stimulation (TMES)

TMES elicits a cervicomedullary evoked potential (CMEP). The stimulation is delivered via adhesive electrodes fixed to the skin over the mastoid processes and current is passed between them, creating a single descending volley. In 1991, Ugawa, Rothwell [98] developed this method to stimulate the descending axons at a subcortical level in order to test spinal excitability (i.e. motoneurons). This single volley contrasts with that of TMS because TMS evokes multiple descending volleys that stimulates corticospinal motoneurons multiple times. The CMEP can be utilized as a measure of motoneurone excitability [99-101]. A fixed latency of the response shows activation of fast descending axons at the level of the pyramidal decussation at the cervicomedullary junction [102, 103]. The stimulation is made possible due to the bending of axons at the decussation, however stimulation at this site is found to be unpleasant. TMES is the most direct method of motoneurone measurement because the descending tracts are not subject to conventional presynaptic inhibition [104, 105]. CMEP however, can have some inconsistencies. If performed incorrectly, activation of peripheral nerve roots independently of spinal stimulation is commonly seen to contaminate CMEP responses. The accepted amount of root activation in CMEP responses exists as percentage of the amplitude of the response. Any activation of peripheral nerves could have an effect on the output that is proposed to be of spinal origin. This interference effects the reliability of the result and could decrease the internal validity of the experiment as there could

be different degree of peripheral activation based on placement of electrodes and anatomic variation of participants. Another issue with TMES is the painful shock needed to elicit the CMEP response. Since it is well known that afferent information has a large effect on corticospinal output, this activation of A-delta and C fibres from pain could hinder the reliability of the results.

2.0.3 Brachial Plexus stimulation

To perform brachial plexus stimulation, Erb's point is electrically excited via adhesive Ag-AgCl electrodes fixed to the skin over the supraclavicular fossa (cathode) and the acromion process (anode). Current pulses can be delivered as a singlet or doublet via a constant current stimulator. The electrical current is gradually increased until the muscle compound action potential (M-wave) of the biceps no longer increases (i.e. Mmax). A supramaximal stimulation current (i.e. 20% higher than that required to elicit Mmax) is often used as a reference for MEPs and CMEPs in testing procedures due to its consistent results. This process accurately accesses the peripheral excitability of the nervous system. The peripheral nerve being stimulated as well as the neuromuscular junction and the muscle itself are not part of the central nervous system however they can alter peripheral excitability as well as overall motor output. [106, 107] Both MEP and CMEP amplitudes or areas are usually expressed as a percentage of Mmax to take into account the effect of muscle excitability on corticospinal excitability. Meaning it allows researchers to eliminate potential differences in excitability of the peripheral and better pinpoint where changes occurred along the corticospinal pathway.

2.0.4 Electromyography

All evoked potentials from TMS, TMES and M-wave responses are recorded and measured from muscle electromyography (EMG). Thus, EMG recording is the primary way corticospinal excitability is measured. Surface EMG recording electrodes are placed 2 cm apart (center to center)

over the mid-point of the muscle belly of the participant. A ground electrode is placed on a point on the body where there is no electrical activity such as the lateral epicondyle. In order to get as accurate results as possible, skin preparation is done for all electrodes, which includes shaving hair off the desired area followed by cleansing with an isopropyl alcohol swab. This allows for decreased skin resistance during recording. An inter-electrode impedance of < 5 kOhms is required prior to recording to ensure an adequate signal-to-noise ratio so that the results obtained are reliable and voided of the majority of false noise. EMG signals, depending on intensity, are usually amplified and filtered to further the reliability of the results.

2.1 The Effect of Pain on Corticospinal Excitability

The change in activity of group III and IV muscle afferents is thought to induce changes in excitability of motoneurons in the spinal cord, or even further up in the motor pathway, such as in the motor cortex (for review see [67]). Group III and IV afferents have been observed to affect motoneurone pools differently in flexors and extensors in cat experiments [108, 109]. When activating afferents involved in nociception through chemical gradient changes, Schomburg et al, found depolarization in motoneurons activating flexors, but in contrast, they found that the motoneurons activating extensors were hyperpolarized.

This phenomenon of flexor excitation and extensor inhibition has been seen before in the literature when looking at the effect of other classes of afferent neurons. These afferents are referred to the flexor reflex afferents [110]. A study by Martin et al. was able to show this same non-uniform effect on motoneurons, previously found in cats, in humans [111]. The researchers were able to find excitation and inhibition in flexor and extensor human motoneurone responses respectively, by directly stimulating the corticospinal tract while in the presence of increased activity of group III and IV muscle afferents, caused by ischemia from fatiguing contractions. It

could be speculated that this reflex occurs in attempt to protect the muscle from pain. In order to pull a limb from harm, it requires that the muscles in the limb be flexed. For this to occur seamlessly, the antagonist, or extensor muscle, must collectively relax. These time sensitive protective measures are usually found to originate spinally, to reduce the processing time that would be required from the motor cortex. With that being said, responses acquired from TMS of the motor cortex have been found to change in the presence of inputs occurring from these group III and IV muscle afferents. Whether the cortically derived responses actually increase or decrease has been disputed in the literature [112, 113]. However, the responses found in these studies were a product of afferents that were excited under different conditions, for example the first study which showed an increase in motor cortex response was found in the presence of hypertonic solution injection in a resting muscle. The second found a decrease in motor output caused by painful injection of ascorbic acid in an active muscle. Therefore, the differences found in corticospinal excitability could have been due to the solution differences or the presence of a resting versus active muscle. The uncertainty could also be due to the idea that muscle responses do not only depend on the motor cortex, as either spinal or peripheral mechanisms may play a role. Thus, further study must be done to assess the effects of these afferents on corticospinal excitability.

More recently, Martin and colleagues have explored how the input of group III and IV muscle afferents differentially effect the output of the motor cortex and motoneurone excitability. To differentiate between the two, they stimulated the motor nerves in the motor cortex using TMS and in the corticospinal tract using TMES. They found a strong feedback from the muscle afferents, which differed depending on what type of exercise was taking place. During fatiguing activity, there was an inhibitory effect of the group III/IV afferents, which contrasted with the results found

during strenuous, non-fatiguing exercise, where group III/IV mediated feedback facilitated motoneuronal excitability without changing excitability to the entire pathway. These results show an increase in spinal excitability with a decrease in supraspinal excitability in the presence of non-fatiguing exercise. However, one could also interpret that the input of these afferents may play a differential role while fatiguing exercise is being performed [114]. This idea was supported further in a paper published by Sidhu and colleagues [115] which investigated lower limb muscle afferent feedback on the excitability of the motor cortex and spinal motoneurone projections to knee extensors during fatiguing and non-fatiguing cycling by attenuating group III/IV muscle afferents using lumbar intrathecal fentanyl. They found during cycling induced leg fatigue activation of III/IV afferents decreased the amount of voluntary descending drive from the motor cortex, which lead to a decline in both flexor and extensor muscle activation. This also caused a depressed excitability of the corticospinal pathway, including the motor cortex and spinal motoneurons in the presence of muscle group III/IV afferent activity [116].

In the presence of non-fatiguing painful exercise, it seems reasonable to see a facilitation of spinal excitability and a decrease in supraspinal excitability. It has been proposed that painful phenomenon is causing a prevention of motor cortex output so that spinal protective mechanisms can take place without interruption [117]. In contrast, while undergoing fatiguing exercise the properties of the afferent feedback interaction must switch. The objective no longer becomes pulling away from danger, instead, the body has to try and protect itself from further injury by reducing motor output as a whole, so that the fatiguing exercise, which is causing hardship, will stop. However, the Sidhu paper found some contrasting results in the presence of non-fatiguing exercise as group III/IV muscle afferents facilitated motor cortical cells, while inhibiting spinal motoneurons [115]. It is important to note that this study did not induce pain through added

stimuli, but instead, they blocked afferent input through the use of an injectable epidural analgesic (fentanyl). As a result, the inconsistency between this paper and the other studies using hypertonic solutions to activate III/IV muscle afferents, could be due to the differential activation of nociceptive versus non-nociceptive subtypes within these groups [118]. The inhibitory effect of the group III/IV afferents through analgesic block was speculated to be modulated through presynaptic inhibition of Ia afferents [119]. Due to the differential nature of the experimental procedure, the idea of spinal reflex takeover (Inhibition of the motor cortex that may allow the spinal motor system to freely respond to noxious stimulation) in the presence of pain may still stand true.

There is still little known about the physiological reason behind most of these interactive mechanisms, however, a study by Mercier et al. [117] attempted to determine if sensorimotor integration, the interaction of pain afferents and motor output, occurred via the process of short afferent inhibition (SAI) in the cortex (for more information on SAI see [86]). Their results showed no significant difference in SAI between the pain and neutral conditions, indicating that the interaction between pain/sensory and motor pathways is not mediated by direct rapid pathways and must involve higher order cognitive areas [120].

2.2 The Effect of Topical Analgesic on Corticospinal Excitability

When adding a topical analgesic such as menthol to the already complex interactions of pain on the nervous system, it can only be speculated what changes may occur. Menthol has been shown to cause a presynaptic release of calcium by activating a family of membrane proteins called transient receptor potential channel M8 (TRPM8). This channel is a sensory molecule expressed on some primary afferent neurons. TRPM8's function is to aid nerve endings in the periphery by sensing cool temperatures [121]. By activating TRPM8 through the use of menthol or even

cooling, you activate a subpopulation of afferents that have these TRP receptors present. Activation stimulates a central synaptic release of glutamate (Glu), This glutamate is thought to bind to Group II or III mGluR receptors on the presynaptic muscle damage-activated nociceptive afferents or possibly postsynaptically on dorsal-horn neurons, which causes attenuation of pain sensation [122]. This attenuation would presumably nullify the effect of the A-delta/C-fibres on the nervous system causing less presynaptic inhibition and in turn increasing motor output even in the presence of nociceptive muscle damage.

2.3 Conclusion

In conclusion, there is little known about the effect of increased nociceptive input on corticospinal excitability, especially the effect of DOMS on corticospinal excitability. DOMS is muscle pain that arises 24-72 hours after exercise involving unfamiliar strenuous muscle loading. DOMS is commonly induced by eccentric muscle contractions and may signify exercise-induced muscle damage including impaired excitation-contraction coupling and injury to cytoskeletal proteins in the sarcomere. During DOMS, muscle pain is transmitted via A-delta and C-fibre afferents (group III/IV afferents) to the central nervous system (CNS). While it has been shown that muscle force production is significantly diminished in DOMS, there is limited evidence on the influence of DOMS on corticospinal excitability or inhibition. One way to reduce the negative effect of DOMS on motor output and perhaps alter any DOMS induced changes in corticospinal excitability is by applying a topical analgesic over the muscle(s) with DOMS. Topical analgesic is thought to blunt the transmission of nociceptive throughout the CNS and in turn may alter corticospinal excitability following the application of it to a muscle experiencing DOMS. However, to date no work has investigated how the application of topical analgesic interacts with DOMS to mediate corticospinal excitability.

CHAPTER 2: CO-AUTHORSHIP STATEMENT

My contributions to this thesis are outlined below:

- i) I recruited all participants and analyzed all data collected for this thesis, with the help of my fellow masters' student Mr. Brandon Collins
- ii) With the assistance of Mr. Brandon Collins student, I collected the experimental data for this thesis.
- iii) I prepared the manuscript and thesis with the help and guidance of my supervisor, Dr. Duane Button and Summer Undergraduate Research Award student Mr. Nicholas Snow.
- iv) Dr. Duane Button provided constructive feedback on the manuscript and thesis.

CHAPTER 3: The interactive effect of DOMS and topical analgesic on corticospinal excitability of the biceps brachii.

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3.1 Abstract

The interactive effect of delayed onset muscle soreness (DOMS) and a topical analgesic on corticospinal excitability was investigated during two experiments. A total of Thirty-two participants completed either *Experiments A* (No DOMS, $n=16$) and *B* (DOMS, $n=16$). For each experiment participants were randomly assigned to two groups: 1) menthol-based topical analgesic gel (Topical Analgesic, $n=8$), or 2) placebo gel (Placebo, $n=8$) group. Prior to the application of gel (pre-gel), as well as 5, 15, 30, and 45 min post-gel, motor evoked potential (MEP) area, latency, and silent period, as well as cervicomedullary MEP (CMEP) and maximal compound motor unit action potential (Mmax) areas and latencies were measured. In addition, pressure-pain threshold (PPT) was measured pre-DOMS and at the same time points in *Experiment B*. In *Experiment A*, neither group showed a significant change for any outcome measure. In *Experiment B*, both groups exhibited a significant increase in PPT from pre-DOMS to pre-gel (Topical Analgesic, pre-DOMS = 7.03 ± 2.48 kg, pre-gel = 3.12 ± 1.26 kg, $p < .001$; Placebo, pre-DOMS = 5.77 ± 2.35 kg, pre-gel = 3.51 ± 1.58 kg, $p < .05$). Following the application of topical analgesic, but not placebo, there was a significant increase in PPT at 15, 30, and 45 min post-gel (3.70 ± 1.69 , 3.92 ± 1.67 and 4.33 ± 1.65 kg, $p < .05$), respectively compared to pre-gel (3.12 ± 1.26 kg) and an increase in silent period at post-30 and 45 min (96.08 ± 26.62 ms and 94.23 ± 22.32 ms, $p < .05$) compared to pre-gel (85.67 ± 19.65 ms). DOMS reduced MEP area (*Experiment A* = 0.222 ± 0.169 ; *Experiment B* = 0.097 ± 0.057 , $p < .02$), CMEP area (*Experiment A* = 0.186 ± 0.148 ; *Experiment B* = 0.077 ± 0.045 ; $p < .05$), and increased MEP silent period (*Experiment A* = 77.41 ± 31.05 ms; *Experiment B* = 100.85 ± 32.29 ms; $p < .05$). These findings suggest that DOMS reduced corticospinal excitability; and following the administration of menthol-based topical analgesic there was a reduction in pain, which was accompanied by increased corticospinal inhibition.

3.2 Introduction

Delayed onset muscle soreness (DOMS) is muscle pain induced through unfamiliar strenuous muscle loading arising 24-72 hours post exercise [30]. Muscles soreness has been shown to activate type III/IV muscle afferent fibres [123] which induces neuromuscular impairments, including altered joint kinematics, a loss in force perception [60], muscular strength [59], joint angle perception, MVC production [59, 61-64], a decrease in muscle activation and increased EMG to force ratios [65]. The excitability of the corticospinal tract, considered the most dominant descending tract for voluntary control of motor output, has been studied under various including experimentally induced muscle soreness through hypertonic saline injection [112] or non-fatiguing ischemic exercise [109]. There are few studies, however, that have assessed corticospinal excitability (CSE) in the presence of DOMS.

One way to quantify CSE is via transcranial magnetic stimulation (TMS) and transmastoid electrical stimulation (TMES). TMS and TMES elicit motor evoked potentials (MEP) and a cervicomedullary MEPs (CMEP), respectively. By using both of these approaches one can assess if changes are of supraspinal or spinal origin along the corticospinal pathway. TMS activates corticospinal neurons leading to the activation of motoneurons. MEPs provide an idea of the excitability of the motor system at the time of the TMS pulse. The amplitude or area of MEPs produced by single-pulse TMS indicates the summation of excitatory and inhibitory inputs to the corticospinal pathway, allowing a measure of CSE to which both supraspinal and spinal excitability contribute [121]. In addition, the cortical silent period (CSP) is a period of dormancy in the rectified EMG trace immediately after a MEP, when TMS is delivered during a sustained tonic contraction of the target muscle contralateral to the motor cortex M1 [124, 125]. It has been suggested that the level of contraction and the size of the MEP do not impact CSP, while its

duration tends to increase linearly with TMS stimulus intensity [126, 127]. Duration of the CSP is thought to be influenced by both cortical and central mechanisms [124]. In the cortex, γ -aminobutyric acid (GABA)-ergic interneurons (i.e., GABA_B receptors) appear to mediate the CSP [128, 129].

Activation of group III and IV muscle afferents has been shown to induce changes in the excitability of motoneurons both spinally and supraspinally (for review see [67]). In cat experiments, researchers have found these afferents differentially influence flexor and extensor motoneurone pools [106, 107]. These experiments activated III/IV muscle afferents involved in nociception by intra-arterial injections of potassium and bradykinin, and the researchers found depolarization in motoneurons leading to activation in flexors, by contrast, motoneurons activating extensors were hyperpolarized leading to inhibition. This phenomenon of flexor excitation and extensor inhibition has been seen before in the literature when looking at the effect of other classes of afferent neurons. These afferents are referred to the flexor reflex afferents [108]. A study by Martin et al. [109] was able to show the same non-uniform effect on motoneurons previously found in cats also occur in humans. The researchers were able to find excitation in flexor and inhibition in extensor motoneurone response, by directly stimulating the corticospinal tract through TMS and TMES in the presence of increased activity of group III and IV muscle afferents, caused by ischemia from fatiguing contractions. Martin et al. [112] also determined how group III and IV muscle afferents activation differentially affected the excitability of the motor cortex and spinal cord, respectively. To differentiate between the two, they stimulated the motor cortex using transcranial magnetic stimulation (TMS) and the corticospinal tract using transmastoid electrical stimulation (TMES). By using hypertonic saline injections to activate III/IV muscle afferents, they showed an increase in spinal excitability with a decrease in supraspinal

excitability without a change in MEP amplitude [112]. Sidhu and colleagues [113] investigated lower limb muscle afferent feedback on the excitability of the motor cortex and spinal motoneurone projections to knee extensors during fatiguing and non-fatiguing cycling by attenuating group III/IV muscle afferents using lumbar intrathecal fentanyl. They found during cycling induced leg fatigue activation of III/IV afferents decreased the amount of voluntary descending drive from the motor cortex, which lead to a decline in both flexor and extensor muscle activation. In a second study, they were able to show depressed excitability of the corticospinal pathway, including the motor cortex and spinal motoneurons to an upper limb muscle during fatiguing leg exercise in the presence of muscle group III/IV afferent activity [114].

In the presence of painful exercise, it seems reasonable to see a facilitation of spinal excitability and a decrease in supraspinal excitability. It has been proposed that painful phenomenon is causing a prevention of motor cortex input so that spinal protective mechanisms can take place without interruption [115]. Sidhu et al., [113] did find contrasting results to support this, in the presence of non-fatiguing exercise, group III/IV muscle afferents facilitated motor cortical cells, while inhibiting spinal motoneurons [113] but during exhausting exercise the motor cortex was inhibited and motoneurons were unaffected. It is important to note that this study did not induce pain through added stimuli, but instead, they blocked afferent input through the use of an injectable epidural analgesic (fentanyl). As a result, the inconsistencies between this paper and the other studies using hypertonic solutions to activate III/IV muscle afferents, could be due to the differential activation of nociceptive versus non-nociceptive subtypes within these groups [116].

A number of studies looking at single motor unit recordings in biceps showed that DOMS increased mean motor unit discharge rates and synchronization, while decreasing motor unit recruitment thresholds [125, 126]. In a study by Vansgaard et al., middle trapezius eccentric

muscle contraction-induced DOMS resulted in increased stimulus intensity needed to achieve baseline H-reflex amplitude, 24 hours post-exercise. This suggested diminished spinal excitability produced by presynaptic inhibition of Ia afferent fibres, the afferent arc of the H-wave, by group III/IV afferents [127]. So there is evidence of some spinal alteration involved in DOMS associated deficits. Despite the above research findings, there is limited evidence on the influence of DOMS on CSE or corticospinal inhibition. Likewise, it is unknown whether CNS effects of DOMS are restricted to changes at the spinal versus supraspinal level [128].

When adding topical analgesic such as menthol to the complex interplay between DOMS and motor output, it can only be speculated what changes may occur. Menthol acts on a subset of transient receptor proteins (TRPs) which are membrane proteins called transient receptor potential channel M8 (TRPM8) [119]. The activation of the afferent fibres through these receptors has been shown to cause a presynaptic release of calcium as well as a central synaptic release of glutamate. This glutamate is thought to bind to Group II or III mGluR receptors on the presynaptic muscle damage-activated nociceptive afferents or possibly post-synaptically on dorsal-horn neurons, which causes attenuation of pain sensation [120]. Thus, a topical analgesic (i.e. menthol) may nullify the effect of the group III/IV muscle afferents on the nervous system causing less presynaptic inhibition and in turn increasing motor output even in the presence of nociceptive muscle damage.

The purpose of this study was to assess the: 1) effect of DOMS on CSE and 2) interactive effect of DOMS and a topical analgesic gel on CSE. We hypothesized that DOMS would result in cortical inhibition and spinal facilitation which would be attenuated by the application of a topical anesthetic.

3.3 Methods

Participants

Sixteen young healthy adult participants were recruited for *Experiment A*: No DOMS and 16 young healthy adult male participants were recruited for *Experiment B*: DOMS. Five participants from *Experiment A* also performed *Experiment B* (see **Table 1** for participant demographics). All participants were recreationally active (~10 hours of activity/week). Prior to testing, all participants completed the magnetic stimulation safety checklist (Rossi, Hallett, Rossini, & Pascual-Leone, 2011) and Physical Activity Readiness Questionnaire (Canadian Society for Exercise Physiology (CSEP), 2002). Additionally, participants were instructed to refrain from heavy exercise 24 hours before testing and to follow the Canadian Society for Exercise Physiology preliminary instructions (i.e., no eating for 2 hours, drinking caffeine for 2 hours, smoking for 2 hours, or drinking alcohol for 6 hours) (CSEP, 2013) prior to the start of testing. Before participating in the study, each participant was initially briefed verbally and in writing as to the risks of the research and was given the opportunity to provide written informed consent, in compliance with the Declaration of Helsinki and the university's institutional ethics review board regulations. The University's Interdisciplinary Committee on Ethics in Human Research approved the study (#20171278-HK), which was in accordance with the Tri-Council guidelines in Canada with full disclosure of potential risks to participants.

DOMS Protocol

In *Experiment B* only, participants performed an eccentric elbow flexor contraction protocol to induce DOMS. For all participants, this protocol was completed using a HUMAC NORM dynamometer (CSMi Medical Solutions, Stoughton, MA, USA). The elbow of the dominant arm was aligned with the rotational axis of the dynamometer. Excess movement was

reduced by using shoulder weights and chest straps. Participants were seated upright with their back supported at a 90° angle and were instructed to complete a comfortable range of motion (~110° degrees of rotation, on average). The desired range of motion was then divided by 5, to establish participants' required degrees per second for the eccentric contraction used during the DOMS protocol. The protocol began with 1 warmup set which consisted of eccentric contractions that were perceived by the participant to be approximately 50% maximum eccentric torque output. Once the warmup set was completed, participants performed the DOMS protocol which consisted of only eccentric contractions for 5 sets of 10 repetitions that were 5 seconds each in duration. Participants were given 90 seconds of rest between each set. Following each eccentric contraction, participants were told to apply no force as the dynamometer passively brought the arm back to full flexion. Participants were instructed to maximally contract for each eccentric contraction.

Elbow Flexor Force

Participants were seated in a custom-built chair (Technical Services, Memorial University of Newfoundland, St. John's, NL, Canada) in an upright position, with the chest and head strapped in place to minimize movement, and the hips and knees flexed at 90°. The shoulder was placed at 0° and the elbow was flexed at 90°. At the 0° position, both arms were slightly abducted and rested on a padded support. The forearm was held horizontal, positioned midway between neutral and supinated positions, and placed in a custom-made orthosis that was connected to a load cell (Omegadyne Inc., Sunbury, OH, USA). The load cell detected force output, which was amplified $\times 1000$ (CED 1902, Cambridge Electronic Design Ltd., Cambridge, UK) and displayed on a computer screen. Data were sampled at 2000 Hz. Participants were instructed to maintain an upright position with their head in a neutral position during contractions of the elbow flexors.

Verbal encouragement and visual feedback (Signal 4.0 software, Cambridge Electronic Design Ltd., Cambridge, UK) were given to all participants during elbow flexor contractions.

Electromyography (EMG)

Participants' EMG activity was recorded using bipolar surface EMG recording electrodes (Meditrace Ag-AgCl pellet electrodes, disc-shaped 10 mm diameter, Graphic Controls Ltd., Buffalo, NY, USA) from the biceps brachii muscle of the dominant arm. Electrodes were placed 2 cm apart (center to center) over the midpoint of the muscle belly of each participant's dominant biceps brachii muscle. A ground electrode was placed over the lateral epicondyle of the ipsilateral knee. Skin preparation for all recording electrodes included shaving the skin to remove excess hair, lightly abrading the skin surface with fine-grit sandpaper to remove excess dry epithelial cells, and cleaning the skin with a 70% isopropyl alcohol swab to eliminate dry epithelial cells. An inter-electrode impedance of $< 5 \text{ k}\Omega$ was obtained prior to recording to ensure an adequate signal-to-noise ratio. All EMG signals were amplified ($\times 1000$) (CED 1902, Cambridge Electronic Design Ltd., Cambridge, UK) and filtered using a 3-pole Butterworth filter with cut-off frequencies of 10-1000 Hz. All signals were analog-to-digital converted at a sampling rate of 5000 Hz using a CED 1401 interface (Cambridge Electronic Design Ltd., Cambridge, UK).

Stimulation Conditions

Transcranial magnetic stimulation (TMS)

TMS-elicited motor evoked potentials (MEPs) were used to measure corticospinal excitability (CSE) in all participants. TMS was delivered using a circular coil (13 cm outside diameter, Magstim Company Ltd., Carmarthenshire, UK) and Magstim 200² magnetic stimulator (maximal output 2.0 T, Magstim Company Ltd., Carmarthenshire, UK). The stimulating coil was positioned directly over the vertex of participants' head to induce MEPs in the relaxed and active

(5% maximal voluntary contraction (MVC)) biceps brachii muscle [93]. The vertex was located by marking the measured halfway points between the nasion and inion and the tragus to tragus. The intersection of these two points was defined as the vertex and was clearly marked with a felt-tipped permanent marker. Electrical currents flowed in an anticlockwise direction through the circular coil. The coil was placed horizontally over the vertex so that the direction of the current flow in the coil preferentially activated the right or left primary motor cortex (“A” side up for right side, “B” side up for left side), for the elicitation of current in of the dominant biceps brachii motor cortical representation [129]. Stimulation intensity was set to elicit a threshold MEP (active motor threshold (AMT)), with the size needing to be discernible from the background EMG at 5% of MVC in 50% of the trials (i.e., 4 out of 8 trials) in the biceps brachii muscle [130]. Stimulator output was then increased to 20% above AMT for the remainder of the experiment (*Experiment A*: No DOMS: $M \pm SD$ stimulation intensity = 46 ± 6 %MSO, range = 36-58 %MSO; *Experiment B*: DOMS: $M \pm SD$ stimulation intensity = 57 ± 8 %MSO, range = 42-74 %MSO) [93]. At each time-point of interest (see Experimental Protocols section below), 10 MEP trials were measured at 20% above AMT.

Transmastoid electrical stimulation (TMES)

TMES was applied via surface electrodes (Meditrace Ag-AgCl pellet electrodes, disc-shaped 10 mm diameter, Graphic Controls Ltd., Buffalo, NY, USA) placed over the mastoid processes with current passed between the electrodes by a constant-current electrical stimulator (square wave pulse, 200 μ s duration; model DS7AH, Digitimer Ltd, Welwyn Garden City, UK). Stimulation intensity was adjusted to prevent ventral root activation by closely monitoring cervicomedullary evoked potential (CMEP) responses for any decrease in onset latency (~ 2 ms), which indicates cervical ventral root activation [93]. Stimulation intensity was adjusted to elicit a

CMEP response that matched the size of the average MEP amplitude (recorded at 20% above threshold) during 5% of MVC (*Experiment A*: No DOMS: $M \pm SD$ stimulation intensity = 121.5 ± 22.2 mA, range = 90.0-155.0; *Experiment B*: $M \pm SD$ stimulation intensity = 129.8 ± 25.5 mA, range = 92.0-176.0 mA). At each time-point of interest (see Experimental Protocols section below), eight CMEP trials were measured at 20% above CMEP threshold.

Brachial plexus stimulation

Stimulation of the brachial plexus was used to measure participants' maximal compound motor unit action potential (Mmax). Erb's point was electrically stimulated via a cathode (Meditrace Ag-AgCl pellet electrode, disc-shaped 10 mm diameter, Graphic Controls Ltd., Buffalo, NY, USA) positioned on the skin overlying the supraclavicular fossa and an anode electrode (Meditrace Ag-AgCl pellet electrodes, disc-shaped 10 mm diameter, Graphic Controls Ltd., Buffalo, NY, USA) placed over the acromion process. Current pulses were delivered as a singlet using a constant-current electrical stimulator (square wave pulse, 200 μ s duration; model DS7AH, Digitimer Ltd, Welwyn Garden City, UK). The electrical current was gradually increased until Mmax of the biceps brachii muscle was reached at rest. The maximal stimulation intensity used to determine Mmax during rest was then used throughout the rest of the experiment (*Experiment A*: No DOMS: $M \pm SD$ stimulation intensity = 112.1 ± 124.3 mA, range = 80.0-165.0 mA; *Experiment B*: $M \pm SD$ stimulation intensity = 139.1 ± 45.4 mA, range = 75.0-255.0 mA). At each time-point of interest (see Experimental Protocols section below), three Mmax trials were measured at the stimulator intensity used to arrive at Mmax.

Pressure-pain threshold (PPT)

In *Experiment B* only, PPT was quantified for each participant's dominant biceps brachii muscle during each session via a pressure-pain algometer. A mark was placed on the participant's biceps brachii muscle at the mid-belly, 9 cm above the antecubital space on the first day of testing to ensure measurements were reliable between testing sessions [131]. The algometer (Lafayette Manual Muscle Test System™, Model 01163, Lafayette Instrument Company, IN, USA) was a handheld muscle tester with a pressure range of 0-136.1 kg, comprised of a padded disc with a surface area of 1.7 cm², attached to a microprocessor-control unit that measured peak pressure (kg). The unit had a digital readout for peak-applied pressure and provided a built-in calibration routine that verified a valid calibration. To determine PPT, the researcher would apply the algometer at a gradual force rate of 50-60 kPa/s to the marked spot on the participant's biceps brachii muscle until the participant verbally informed the researcher when the sensation of pressure became painful [132]. At the point of pain, the pressure-pain algometer was removed and the PPT value was recorded. PPT values were obtained every 30 seconds over the tender spot using the pressure-pain algometer at each time-point of interest (see Experimental Protocols section below).

Experimental Protocols

For *Experiment A* and *Experiment B* participants were randomly allocated to one of two groups: 1) topical analgesic gel (Topical Analgesic, $n = 8$ for each experiment); or 2) placebo gel (Placebo, $n = 8$ for each experiment) (**Table 1**). Throughout the experiments, both the experimenter and participant were blinded to the group allocation and gel type of each participant. Each participant was assigned an unmarked tube of gel that contained only a randomization code. The identity of the code was unknown to the experimenter who interacted with the participants during the application of gel. For *Experiment A*, all participants were required to attend one testing session

for approximately 1.5-2 hours. For *Experiment B*, all participants were required to attend two testing sessions, with the first session lasting approximately 45 minutes and the second session lasting approximately 1.5-2 hours. In both experiments either 2 mL of menthol-based topical analgesic gel (Biofreeze®, containing 3.5% menthol; Topical Analgesic) or 2 mL of placebo gel (containing the same ingredients as Biofreeze®, except menthol; Placebo), were applied topically over the belly of the dominant biceps brachii muscle. During *Experiment A* this occurred in the single testing session; for *Experiment B*, gel was applied during the second testing session. The gel was gently applied by an experimenter, and the mode of application for the gels did not involve substantial force, pressure, or rubbing. The same experimenter applied the gel for each participant and the method of application did not differ across groups. The dose of gel (2 mL) was based upon the estimate that the average skin surface area over the biceps brachii was approximately 400 cm², as well as the recommended dosage of Biofreeze® of 1 mL per 200 cm² of skin surface area [82, 133]

Experiment A protocol

During the experimental session participants were familiarized with the different stimulations they would receive (i.e., TMS, TMES, brachial plexus stimulation; see Stimulation conditions section). Participants were positioned to perform an elbow flexion MVC throughout all stimulation procedures (see Elbow Flexor Force section). Participants then completed two elbow flexors MVCs, which were required to have force measurements (N) within 5% of one another to ensure maximal force output; if not, a third MVC was performed. The MVCs were preceded by a 10-minute rest period where the participants were prepped for EMG and stimulation conditions. Following 10 minutes of rest the stimulation intensities were set. Participants' CSE measurements of the biceps brachii muscle (i.e., MEP, CMEP, Mmax) were taken pre-, as well as 5, 15, 30, and

45 minutes post-application of the gel. All stimulations were performed during a biceps brachii of the dominant upper-limb at 5% MVC. Biceps brachii contractions lasted for 80 seconds, to allow for the randomized recording of 10 MEPs, eight CMEPs, and three Mmax trials. All MEP and CMEP stimulations occurred at a frequency of 0.25 Hz; Mmax stimulations were delivered 2 seconds randomly after a MEP recording. (See Figure 1a for experimental set up)

Experiment B protocol

During experimental session one, participants were familiarized with the different stimulations they would receive. Then PPT was measured (see Pressure-pain threshold (PPT) section). Following these measurements, the participants completed the DOMS protocol (see DOMS Protocol section). Participants were then instructed to come back to the laboratory 48 hours later to complete experimental session two. In experimental session two, participants completed the exact same stimulation protocol as in *Experiment A* (see *Experiment A* protocol section). In addition to the pre-DOMS measurement, PPT was recorded prior to, as well as 5, 15, 30, and 45 minutes following the application of the gel, which took place after the CSE measurements at each time point. (See Figure 1b for experimental set up)

Data Analysis

During the DOMS protocol, torque was measured for each repetition and peak torque was extracted for each set (see DOMS Protocol above). For measures of CSE (i.e., MEP, CMEP, Mmax), area was measured between cursors set from the initial EMG deflection from baseline to the second crossing of the horizontal axis [134]. MEP and CMEP areas were extracted in millivolts \times seconds (mV \cdot s) units. The average of all respective MEP ($n = 10$) and CMEP ($n = 8$) areas were normalized to the recorded Mmax ($n = 3$) area values within the same time point (i.e., pre-gel, post-5 min, post-15 min, post-30 min, post-45 min); MEP area and CMEP area data reported in

the results section were expressed as ratios relative to Mmax area. Mmax is a stable measure of muscle activity during maximal muscle fibre recruitment [135] and was thus used as the reference to normalize measures of CSE [136]. For MEPs, the duration of the silent period was considered as the time lapse between MEP offset and the re-onset of muscle activity [137, 138]. Silent period was extracted in ms units and all 10 MEP trials were averaged. Additionally, the latency of MEPs, CMEPs, and Mmax measurements were also extracted in ms as the duration from the stimulus artifact to the initial deflection from baseline and were averaged across the total number of stimulation trials (MEP, $n = 10$; CMEP, $n = 8$, Mmax, $n = 3$). All torque and CSE data were measured offline using Signal 4.0 software (Cambridge Electronic Design Ltd., Cambridge, UK), and averages and ratios were calculated using Office Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

Demographic information used to characterize the study sample in both experiments included age (yr), body mass (kg), and height (cm) (**Table 1**). In *Experiment A*, sex was also recorded because the sample was comprised of both females and males, whereas the sample in *Experiment B* consisted of males only. In *Experiment B*, decrease in peak torque (%) from the first to the last set of the DOMS protocol was reported. Percent-changes in peak torque were calculated by extracting the difference between peak torque values ($\text{N}\cdot\text{m}$) from the first and final sets of the DOMS protocol, relative to the peak torque value of the first set. This value was used to quantify the resistance employed in the DOMS protocol across groups. Neurophysiological outcome measures (i.e., CSE) considered in both experiments included MEP area/Mmax area ratio, MEP latency (ms) and silent period (ms), CMEP area/Mmax area, CMEP latency (ms), Mmax area ($\text{mV}\cdot\text{s}$), and Mmax latency (ms). PPT (kg) was also reported in *Experiment B*.

Prior to statistical analyses all data underwent quality control checks for missing datapoints and outliers. There were no missing data points in *Experiment A*. In *Experiment B*, PPT data were absent for one participant (P08) at all time-points (pre-DOMS, pre-gel; 5, 15, 30, and 45 min post-gel) due to an equipment (i.e., pressure-pain algometer) malfunction. Two additional participants in *Experiment B* (P04, P07) did not have pre-DOMS PPT data for the same reason. These missing data points were imputed by determining the series average for the entire sample of *Experiment B* (including the Topical Analgesic and Placebo groups) at their respective time-points using the Missing Values Analysis and Transform functions in SPSS (V25.0, IBM Corporation, Armonk, NY, USA) (Gamst, Meyers, & Guarino, 2008). Outliers were considered data points that exceeded the sample mean of either respective experiment by \pm three standard deviations (SD) (Portney & Watkins, 2009). In *Experiment A* raw data, one MEP area (0.1%), two CMEP latency (0.3%), and one Mmax latency (0.4%) trials were deemed outliers and were omitted. For *Experiment B* no outlier values were identified. Outlier analysis was performed using Microsoft Office Excel 2016 (Redmond, WA, USA).

Statistical Analysis

Statistical analyses were completed using SPSS. Assumptions of normality (Shapiro-Wilk test), sphericity (Mauchly's test), and homogeneity of variances (Levene's test) were tested for all outcome measures, where appropriate. All data were deemed normally distributed. In the event of a violation of the assumption of sphericity, *p*-values were adjusted using the Greenhouse-Geisser correction. If the assumption of homogeneity of variances was violated, *p*-values were adjusted (equal variances not assumed). These instances are indicated in the text below (see Results section).

Pre-gel values of all outcome measures and demographics were compared across groups using independent-samples *t*-tests, to ensure groups were well-matched at baseline. In *Experiment A*, the proportions of female and male participants in each group were compared using the Pearson χ^2 test. For *Experiment B*, pre-DOMS and pre-gel values of PPT were compared across groups (Topical Analgesic, Placebo) using independent-samples *t*-tests to ensure matching at the study outset, as well as following the DOMS protocol, respectively. To establish that the DOMS protocol induced DOMS in both participant groups (Topical Analgesic, Placebo), pre-DOMS and pre-gel PPT values were compared within each respective group using paired-samples *t*-tests. To examine the effect of topical analgesic on CSE measures (*Experiment A*, *Experiment B*) and PPT (*Experiment B*), one-way repeated-measures analyses of variance (rmANOVAs) (Factor = TIME [pre-gel, and 5, 15, 30, and 45 min post-gel]) were performed on all outcome measures within each group (Topical Analgesic, Placebo) [139]. Any significant main effect of TIME was explored using paired-samples *t*-tests and Fisher's Least Significant Difference adjustment.

Finally, across *Experiment A* versus *Experiment B*, grand means of baseline data (with topical analgesic and placebo groups pooled) were compared to examine whether DOMS had a statistically significant effect on measures of CSE. To ensure the experimental samples were matched at baseline, participants' age, height, and body mass were compared with independent-samples *t*-tests; proportions of female and male participants were compared using the Pearson χ^2 test. Additionally, to determine whether participants' sex influenced baseline statistical comparisons across experiments, analyses of covariance (ANCOVAs) were performed on all CSE data using the fixed factor EXPERIMENT (No DOMS, DOMS) and the covariate SEX (Female, Male). For main statistical tests, statistical significance was set at a level of $p < .05$. Subsequently, raw data are reported as mean (M) \pm SD in the text and tables. In figures, data are expressed as

ratios relative to pre-gel values (post-5 min/pre-gel, post-15 min/pre-gel, post-30 min/pre-gel, post-45 min/pre-gel), and are reported as $M \pm$ standard error of the mean ($SEM = SD/\sqrt{n}$).

3.4 Results

Experiment A: No DOMS

Baseline data

Between-group comparisons of baseline demographics and outcome measures can be found in **Table 1** (demographics) and **Table 2** (outcome measures), respectively. Across groups, there were no significant differences in demographics (age, mass, height, sex), indicating that groups were well-matched (age, mass, height, $t_{(14)} \leq |0.348|$, $p \geq .545$; sex, $\chi^2_{(1)} = 1.000$, $p = .317$). For baseline CSE measures, groups were not significantly different at baseline ($t_{(14)} \leq |1.470|$, $p \geq .121$).

Motor evoked potential (MEP)

See **Figure 2A, 2C**, and **Table 2** for MEP area/Mmax area ratios and MEP latencies and silent periods data. There was no significant main effect of TIME for either the topical analgesic ($F_{(4, 28)} = 0.795$, $p = .538$; $F_{(4, 28)} = 0.942$, $p = .454$; $F_{(4, 28)} = 0.534$, $p = .712$) or placebo ($F_{(4, 28)} = 1.068$, $p = .391$; $F_{(4, 28)} = 2.345$, $p = .079$; $F_{(4, 28)} = 1.328$, $p = .284$) groups on MEP area/Mmax area ratios (**Figure 1A**), MEP latencies (**Table 2**), or silent periods (**Figure 1C**), respectively.

Cervicomedullary evoked potential (CMEP)

See **Figure 3A** and **Table 2** for CMEP area/Mmax area ratios and CMEP latencies data. There was no significant main effect of TIME for either the topical analgesic ($F_{(4, 28)} = 2.156$, $p = .100$; $F_{(4, 28)} = 0.990$, $p = .429$) or placebo ($F_{(4, 28)} = 1.358$, $p = .287$; $F_{(4, 28)} = 0.821$, $p = .424$) groups on CMEP area/Mmax area ratios (**Figure 3A**) or CMEP latencies (**Table 2**), respectively.

Maximal compound motor unit action potential (Mmax)

See **Table 2** for Mmax areas and latencies data. There was no significant main effect of TIME for either the topical analgesic ($F_{(4, 28)} = 0.756, p = .562$; $F_{(4, 28)} = 1.067, p = .339$) or placebo ($F_{(4, 28)} = 1.068, p = .391$; $F_{(4, 28)} = 0.537, p = .535$) groups on Mmax areas or latencies, respectively (**Table 2**).

Experiment B: DOMS

Baseline data

Between-group comparisons of baseline demographics and outcome measures can be found in **Table 1** (demographics) and **Table 3** (outcome measures), respectively. Across groups, there were no significant differences in demographics (age, mass, height) or DOMS protocol resistance (% decrease in peak torque), indicating that groups were well-matched ($t_{(14)} \leq |0.208|, p \geq .267$). In terms of baseline outcome measures, groups were not significantly different at baseline ($t_{(14)} \leq |0.064|, p \geq .184$); however, for silent period and CMEP area/Mmax area, there were trends towards significance (silent period, $t_{(14)} = -2.078, p = .057$; CMEP area/Mmax area, $t_{(14)} = -2.039, p = .061$) whereby these values tended to be greater in the placebo group (silent period = 116.03 ± 36.36 ms; CMEP area/Mmax area = 0.098 ± 0.044) versus the topical analgesic group (silent period = 85.67 ± 19.65 ms; CMEP area/Mmax area = 0.056 ± 0.039). In terms of PPT, groups were not significantly different either prior to the commencement of the DOMS protocol (pre-DOMS, $t_{(14)} = 1.042, p = .315$) or following the DOMS protocol (pre-gel, $t_{(14)} = 0.927, p = .370$). However, for both groups there was a significant decrease in PPT following the inducement of DOMS (topical analgesic, pre-DOMS = 7.03 ± 2.48 kg, pre-gel = 3.12 ± 1.26 kg, $t_{(7)} = -8.470, p = .000063$; placebo, pre-DOMS = 5.77 ± 2.35 kg, pre-gel = 3.51 ± 1.58 kg, $t_{(7)} = -4.013, p = .005$).

Motor evoked potential (MEP)

See **Figure 2B, 2D**, and **Table 3** for MEP area/Mmax area ratios and MEP latencies and silent periods data. There was no significant main effect of TIME for the topical analgesic ($F_{(4, 28)} = 1.403, p = .276$; $F_{(4, 28)} = 0.696, p = .601$) group or the placebo ($F_{(4, 28)} = 1.1594, p = .204$; $F_{(4, 28)} = 2.124, p = .104$) group on MEP area/Mmax area ratios (**Figure 2B**), or MEP latencies (**Table 3**), respectively.

There was, however, a significant main effect of TIME for the topical analgesic group ($F_{(4, 28)} = 3.194, p = .028$) on silent periods (**Figure 2D**). *Post hoc* analyses showed that, compared to pre-gel values (85.67 ± 19.65 ms), there was a non-significant trend towards an increase in silent period at 5 min (93.20 ± 28.14 ms, $t_{(7)} = -2.005, p = .085$), which returned to near-baseline at 15 min (83.57 ± 16.41 ms, $t_{(7)} = 0.453, p = .665$) post-gel application. However, at 30 min (96.08 ± 26.62 ms, $t_{(7)} = -2.430, p = .045$) and 45 min (94.23 ± 22.32 ms, $t_{(7)} = -2.726, p = .029$) post-gel application, there was a significant increase in silent period compared to pre-gel (**Figure 2D, Table 3**). There was no such main effect for TIME on silent period for the placebo group ($F_{(4, 28)} = 0.655, p = .497$) (**Figure 2D**).

Cervicomedullary evoked potential (CMEP)

See **Figure 3B** and **Table 3** for CMEP area/Mmax area ratios and CMEP latencies data. There was no significant main effect of TIME for either the topical analgesic ($F_{(4, 28)} = 2.156, p = .100$; $F_{(4, 28)} = 0.629, p = .646$) or placebo ($F_{(4, 28)} = 0.761, p = .560$; $F_{(4, 28)} = 0.559, p = .563$) groups on CMEP area/Mmax area ratios (**Figure 3B**) or CMEP latencies (**Table 3**), respectively.

Maximal compound motor unit action potential (Mmax)

See **Table 3** for Mmax areas and latencies data. There was no significant main effect of TIME for either the topical analgesic ($F_{(4, 28)} = 2.252, p = .089$; $F_{(4, 28)} = 0.696, p = .601$) or placebo ($F_{(4, 28)} = 0.495, p = .740$; $F_{(4, 28)} = 0.917, p = .393$) groups on Mmax areas or latencies, respectively (**Table 3**).

Pressure-pain threshold (PPT)

See **Figure 4** and **Table 3** for PPT data. There was a significant main effect of TIME for the topical analgesic group ($F_{(4, 28)} = 8.9681.358, p = .000086$). *Post hoc* analyses showed PPT values significantly increased at 15 (3.70 ± 1.69 kg, $t_{(7)} = -2.619, p = .034$), 30 (3.92 ± 1.67 kg, $t_{(7)} = -3.987, p = .005$), and 45 min (4.33 ± 1.65 kg, $t_{(7)} = -4.566, p = .003$) post-gel application compared to pre-gel (3.12 ± 1.26 kg) (**Figure 3**). A significant main effect of TIME was not observed for PPT values ($F_{(4, 28)} = 1.802, p = .156$) in the placebo group (**Figure 3**).

Comparison between *Experiment A*: No DOMS and *Experiment B*: DOMS

Results from baseline comparisons across experiments can be found in **Table 1** (demographics), **Table 4** (outcome measures), and **Figure 5** (outcome measures). In terms of baseline demographic characteristics, participants in *Experiment B*: DOMS were significantly older (*Experiment A*, age = 23 ± 1.1 yr; *Experiment B*, age = 26 ± 5.1 yr; $t_{(30)} = -2.277, p = .037$), taller (*Experiment A*, height = 174.2 ± 8.2 cm; *Experiment B*, height = 179.8 ± 6.1 cm; $t_{(30)} = -2.213, p = .035$), and more massive (*Experiment A*, mass = 71.9 ± 9.1 kg; *Experiment B*, mass = 81.9 ± 9.1 kg; $t_{(30)} = -3.108, p = .004$) than participants from *Experiment A*: No DOMS (**Table 1**). Regarding distribution of participants' sex across samples there was a significantly greater proportion of females in *Experiment A*: No DOMS ($n = 8$) versus *Experiment B*: DOMS ($n = 0$; $\chi^2_{(1)} = 10.667, p = .001$) (**Table 1**).

With reference to baseline (pre-gel) outcome measures, participants in *Experiment B*: DOMS demonstrated significantly smaller values of MEP area/Mmax area (*Experiment A*, 0.222 ± 0.169 ; *Experiment B*, 0.097 ± 0.057 ; $t_{(30)} = 2.821$, $p = .011$) (**Figure 5A**) and CMEP area/Mmax area (*Experiment A*, 0.186 ± 0.148 ; *Experiment B*, 0.077 ± 0.045 ; $t_{(30)} = 2.814$, $p = .012$) (**Figure 5B**), as well as a significantly longer silent period (*Experiment A*, 77.41 ± 31.05 ms; *Experiment B*, 100.85 ± 32.29 ms; $t_{(30)} = -2.093$, $p = .045$) (**Figure 5C**), compared to those in *Experiment A*: No DOMS (**Table 4**). Mmax area exhibited a nonsignificant trend towards a greater value in *Experiment B*: DOMS versus *Experiment A*: No DOMS ($t_{(30)} = -1.733$, $p = .093$) (**Table 4**). Neither MEP latency, CMEP latency, nor Mmax latency showed a statistically significant difference across experiments at baseline ($t_{(30)} \leq |1.697|$, $p \geq .100$) (**Table 4**). ANCOVA results revealed that the covariate SEX was not statistically significant in any analyses of outcome measures ($F_{(2, 29)} \leq 2.253$, $p \geq .144$), suggesting that the uneven distribution of females and males across experiments did not influence differences in outcomes pre-gel.

3.5 Discussion

This study determined the effect of delayed onset muscle soreness and the application of a menthol-based topical analgesic gel (Biofreeze®) on indices of corticospinal excitability (MEP, CMEP), inhibition (silent period), peripheral motor nerve excitability (Mmax), and pressure-pain threshold in the dominant biceps brachii muscle 48 hours post eccentric exercise. By comparing baseline data between two experiments (DOMS and No DOMS) we show that the DOMS induced activation of III/IV muscle afferents inhibited and/or dis-facilitated supraspinal and spinal excitability as well as increased inhibition in motor output. Following the application of an analgesic gel in the presence of DOMS there was a significant increase in the cortical silent period at the 30 and 45 minute time points. These findings were not observed either in persons with

DOMS for whom placebo gel was applied or in participants in whom DOMS was not induced. Furthermore, the application of the topical analgesic gel also altered pain caused by DOMS since we found a significant reduction in perceived pain at the 15,30 and 45-minute time points, a finding that did not occur in the placebo group.

DOMS causes inhibition of the corticospinal pathway to the bicep brachii during active contraction.

DOMS has been used as an experimental model of acute muscle pain (Lau et al., 2015), and represents exercise-induced muscle damage including impaired excitation-contraction coupling and injury to cytoskeletal proteins in the sarcomere [140, 141]. DOMS is muscle pain likely brought on by the generation of inflammatory substances such as bradykinin, potassium, serotonin, histamine, hydrogen ions, and prostaglandins, and transmitted via A-delta and C-fibre afferents (group III/IV afferents) to the CNS [141]. The significant decrease in MEP/Mmax area as well as CMEP/Mmax area represents an inhibition of the motor pathway at the supraspinal and spinal regions in the presence of DOMS. A study by Le Pera, et al., [110] showed similar results with a reduction in MEP amplitude as well as H-reflex amplitude, indicating reduction in both CSE and spinal excitability, in the presence of injected hypertonic saline into the abductor digiti minimi (ADM) muscle. Additional work by the same group showed that DOMS elicited by eccentric middle trapezius contraction required an increased stimulus intensity to reach the baseline H-reflex amplitude 24 hours post-exercise, suggesting a reduction in spinal excitability. It was postulated that group III/IV afferent activation by DOMS resulted in presynaptic inhibition of Ia afferent reflex fibre inputs to spinal motoneurons [127]. However, these results contrast other studies that also assessed both CSE and spinal excitability in flexor muscles, in which the presence of type III and IV afferent activation, caused a facilitation in excitability of spinal

motoneurons and an inhibition of cortical cells [109, 112]. It could be speculated that this facilitation occurs in attempt to protect the muscle from pain. In order to protect a limb from potential injury, generally the muscles in the limb are rapidly flexed, and for this to occur quickly, the antagonist, or extensor muscle, must concomitantly relax. These time sensitive protective measures are usually found to originate spinally, possibly to reduce the processing time that would be required from the motor cortex. However, responses acquired from TMS of the motor cortex have also been found to be altered with the presence of activation of these group III and IV muscle afferents. With activation, whether the motor output is increased or decreased has been disputed in the literature [110, 111]. However, the responses found in these studies were a product of afferent stimulation under different conditions, with the increase in motor cortex response occurring after hypertonic solution injection in a resting muscle and the decrease associated with painful injection of ascorbic acid in an active muscle. Therefore, the differences found in CSE could have been due to the solution differences or the presence of a resting versus active muscle or potentially a combination of both. The uncertainty could also be due to the idea that muscle responses do not only depend on the motor cortex, as either spinal or peripheral mechanisms may play a role.

Other studies have found no change in any measures of CSE attributable to activation of these afferent fibres [131]. However, these studies activated type III/IV afferents through hypertonic saline injection or non-fatiguing ischemic exercise. There would be more structural damage at the location of the muscle during delayed onset muscle soreness as well as less local pain stimulus. Sidhu et al. [113, 114] have shown how these same type III/IV afferents play a role in inhibiting the motor cortex during fatiguing exercise whereas during non-fatiguing exercise they cause excitation. This indicates a differential influence of these afferent fibres on the nervous

system during differing conditions, which may also be at play during delayed onset muscle soreness.

In the current study, DOMS significantly increased the cortical silent period compared to no DOMS. This suggests in the presence of DOMS or type III/IV afferent activation there is an increase in intracortical inhibition. These results may lead to some support of the conclusions by Mercier et al., [118] when they attempted to determine if sensorimotor integration, the interaction of pain afferents and motor output, occurred via the process of short afferent inhibition (SAI) in the cortex. Briefly, SAI is peripheral nerve stimulation paired with single TMS activation of the motor cortex, resulting in an inhibition of TMS activation of muscle response for a few milliseconds after the sensory volley reaches the cortex. Their results showed cutaneous heat pain applied to the hand resulted in decreased cortical excitability but did not result in any significant difference in SAI between the pain and neutral conditions, indicating that the interaction between pain/sensory and motor pathways is not mediated by direct rapid pathways and must involve higher order integrative areas [118]. Recent findings on DOMS have determined that at 48 hours post-exercise, Mmax amplitude in the biceps brachii muscle, elicited by brachial plexus stimulation were no different than pre-DOMS levels [128]. This supports that DOMS related consequences are not likely related to reductions in action potential generation and propagation in the motor unit [142]. Similarly, our findings resulted in no significant difference in Mmax area between DOMS and no DOMS. Thus, there appears to be a strong corticospinal contribution to DOMS-related impairments.

Topical Analgesic gel reduces pain 15, 30 and 45 minutes' post application

A significant difference from pre-values was found at the 15,30 and 45-minute post gel application time points for the pressure-pain threshold measurement for the topical analgesic group. This difference was nonexistent in the placebo group and establishes support for menthol (the medicinal ingredient in the analgesic gel) as a topical analgesic substance that helps to reduce perceived pain. We hypothesized that this change in pain measures would be consistent with alterations in CSE. Similarly, Johar et al. [82] found that menthol significantly reduced DOMS compared to ice application, and that tetanic force was higher in the menthol group compared to the ice group, probably due to a decreased pain sensation following the application of the topical analgesic. This reduction in perceived pain would be due to the activation of the main receptor of menthol, (TRMP8) [132] on a subpopulation of afferents that have TRP receptors present. Activation stimulates a central synaptic release of glutamate (Glu) from these TRP specific afferents, this glutamate is thought to bind to Group II or III mGluR receptors on the presynaptic muscle damage-activated nociceptive afferents or possibly postsynaptically on dorsal-horn neurons, which causes attenuation of pain sensation [120]. This attenuation would presumably nullify the effect of the A-delta/c fibres on the nervous system causing less presynaptic inhibition, and in turn, increase motor output even in the presence of nociceptive muscle damage. However, this does not seem to be the case, it is possible the TRMP8 cascades through a different pathway of afferents to cause analgesia or the Type III/IV afferents have differing inhibition properties while mGluR receptors are bound. Our results showed no excitation and slight indication of inhibition of neurophysiological properties in cortical regions due to the analgesic gel in the presence of DOMS. Furthermore, in *experiment A* (No DOMS) no significant differences in neurophysiological measures were found across any time point throughout the experiment,

indicating a lack of effect on the analgesic gel on cortical or spinal excitability when there is no DOMS present.

The application of topical analgesic to a biceps brachii with DOMS increases intracortical inhibition.

Cortical silent period significantly increased 30 minutes and 45 minutes after the application of analgesic gel, this increase was not present in either the placebo group in *experiment B* or either group in *experiment A*. In terms of its underlying physiology, the CSP is believed to be generated by both spinal inhibitory mechanisms (e.g., recurrent inhibition, refractoriness of spinal motoneurons, post-synaptic inhibition) and inhibitory intracortical interneurons within M1 [130, 143]. Previously, it has been accepted that the duration of the CSP is influenced primarily by cortical mechanisms [130]. In particular, γ -aminobutyric acid (GABA)-ergic interneurons in the cortex (i.e., GABAB receptors) were thought to mediate the CSP [144, 145]. However, a recent study by Yacyshyn et al. has discovered a substantial spinal influence on CSP. This would indicate that only in the presence of DOMS/type III/IV afferent activation, does the stimulation of TRPM8 receptors cause an increased nervous system inhibition either spinally or cortically. We proposed that the activation of these receptors would cause an increase in motor output due to less afferent input from the nociceptive fibres, but in fact, this interaction caused further inhibition while still decreasing pain perception. This would indicate a clear interaction of the afferent fibres affected by menthol and those through which DOMS act on.

Conclusion

In conclusion DOMS had an inhibitory effect on both supraspinal and spinal derived motor output when compared to the no DOMS group. Furthermore, the application of the topical analgesic gel resulted in increased pressure-pain threshold, indicating a reduced pain perception at

15, 30 and 45 minutes' post application and increased cortical inhibition, as measured by an increased CSP, at 30 and 45 minutes post application. These changes only occurred in the presence of DOMS. The application of topical analgesic in the absence of DOMS resulted in no significant effect on CSE, indicating that the topical analgesic has an effect on the central nervous when applicant is experiencing DOMS and helps alleviate the individual's DOMS associated perceived pain.

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3.7 References

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Tables

Table 1. Baseline demographic values. Data are expressed as Mean (Standard Deviation).
*, statistically significant difference, $p < .05$.

Experiment A: No DOMS					
Demographic	Topical Analgesic (n = 8)		Placebo (n = 8)		p-value (Topical Analgesic vs. Control)
Age (yr)	25	(6.4)	26	(3.9)	.545
Mass (kg)	79.3	(8.1)	84.5	(9.8)	.619
Height (cm)	180.2	(6.1)	179.5	(6.5)	.340
Sex (n)	F = 3	M = 5	F = 5	M = 3	.317
Experiment B: DOMS					
Demographic	Topical Analgesic (n = 8)		Placebo (n = 8)		p-value (Topical Analgesic vs. Control)
Age (yr)	25	(6.4)	26	(3.9)	.710
Mass (kg)	79.3	(8.1)	84.5	(9.8)	.267
Height (cm)	180.2	(6.1)	179.5	(6.5)	.838
Decrease in Torque (%)	34.9	(22.7)	31.1	(21.1)	.735
Experiment A: No DOMS Versus Experiment B: DOMS					
Demographic	Experiment A - No DOMS (n = 16)		Experiment B - DOMS (n = 16)		p-value (Experiment A vs. Experiment B)
Age (yr)	23	(1.1)	26	(5.1)	*.037
Mass (kg)	71.9	(9.1)	81.9	(9.1)	*.004
Height (cm)	174.2	(8.2)	179.8	(6.1)	*.035
Sex (n)	F = 8	M = 8	F = 0	M = 16	*.001

Table 2. Raw data values for neurophysiological outcome measures in *Experiment A*: No DOMS. The *p*-value column indicates between-group (topical analgesic, *n* = 8; control, *n* = 8) comparisons of baseline outcome measures. MEP, motor evoked potential; Mmax, maximal compound motor unit action potential; CMEP, cervicomedullary evoked potential.

Outcome Measure	Group 1 - Topical Analgesic (<i>n</i> = 8)		Group 2 - Control (<i>n</i> = 8)		<i>p</i>-value (Pre-)
MEP Area (MEP/Mmax Ratio)	Mean	(SD)	Mean	(SD)	
Pre-Gel	0.243	(0.195)	0.202	(0.150)	.647
5 min Post-Gel	0.211	(0.144)	0.192	(0.171)	—
15 min Post-Gel	0.239	(0.207)	0.185	(0.150)	—
30 min Post-Gel	0.170	(0.102)	0.210	(0.186)	—
45 min Post-Gel	0.174	(0.097)	0.170	(0.119)	—
MEP Latency (ms)					
Pre-Gel	12.29	(1.73)	11.69	(1.35)	.455
5 min Post-Gel	11.74	(1.06)	10.59	(1.91)	—
15 min Post-Gel	11.72	(0.68)	11.22	(1.66)	—
30 min Post-Gel	11.41	(0.73)	10.80	(2.39)	—
45 min Post-Gel	11.69	(0.95)	12.43	(1.28)	—
Silent Period (ms)					
Pre-Gel	89.74	(19.44)	65.08	(36.60)	.115
5 min Post-Gel	92.14	(17.96)	60.66	(26.24)	—
15 min Post-Gel	95.63	(20.30)	71.37	(43.72)	—
30 min Post-Gel	91.48	(18.57)	76.01	(32.03)	—
45 min Post-Gel	94.90	(19.57)	69.19	(41.39)	—
CMEP Area (CMEP/Mmax Ratio)					
Pre-Gel	0.200	(0.172)	0.172	(0.130)	.717
5 min Post-Gel	0.148	(0.086)	0.125	(0.075)	—
15 min Post-Gel	0.146	(0.107)	0.122	(0.079)	—
30 min Post-Gel	0.127	(0.086)	0.134	(0.075)	—
45 min Post-Gel	0.124	(0.082)	0.137	(0.099)	—
CMEP Latency (ms)					
Pre-Gel	7.44	(1.33)	7.85	(1.34)	.943
5 min Post-Gel	7.76	(0.87)	8.36	(2.08)	—

15 min Post-Gel	7.58	(1.01)	8.37	(2.26)	—
30 min Post-Gel	7.95	(1.32)	8.20	(1.76)	—
45 min Post-Gel	7.72	(1.24)	8.21	(1.18)	—
Mmax Area (mV·s)					
Pre-Gel	0.07	(0.06)	0.07	(0.03)	.920
5 min Post-Gel	0.08	(0.06)	0.08	(0.04)	—
15 min Post-Gel	0.08	(0.06)	0.08	(0.04)	—
30 min Post-Gel	0.08	(0.06)	0.07	(0.04)	—
45 min Post-Gel	0.08	(0.06)	0.08	(0.04)	—
Mmax Latency (ms)					
Pre-Gel	4.21	(0.98)	5.30	(1.86)	.164
5 min Post-Gel	5.57	(3.39)	5.14	(1.32)	—
15 min Post-Gel	4.36	(1.06)	5.29	(1.28)	—
30 min Post-Gel	4.45	(1.05)	5.40	(1.87)	—
45 min Post-Gel	4.40	(0.94)	4.92	(2.46)	—

Table 3. Raw data values for neurophysiological outcome measures and pressure pain threshold in *Experiment B*: DOMS. The *p*-value column indicates between-group (topical analgesic, *n* = 8; control, *n* = 8) comparisons of baseline outcome measures. MEP, motor evoked potential; Mmax, maximal compound motor unit action potential; CMEP, cervicomedullary evoked potential; PPT, pressure pain threshold; DOMS, delayed-onset muscle soreness. *, statistically significant difference from pre-gel value (*p* < .05). †, statistically significant difference from pre-DOMS value (*p* < .05).

Outcome Measure	Group 1 - Topical Analgesic (<i>n</i> = 8)		Group 2 - Control (<i>n</i> = 8)		<i>p</i> -value (Pre-)
MEP Area (MEP/Mmax Ratio)	Mean	(SD)	Mean	(SD)	
Pre-Gel	0.075	(0.036)	0.118	(0.068)	.132
5 min Post-Gel	0.092	(0.060)	0.150	(0.076)	—
15 min Post-Gel	0.089	(0.049)	0.140	(0.075)	—
30 min Post-Gel	0.088	(0.055)	0.134	(0.074)	—
45 min Post-Gel	0.081	(0.044)	0.136	(0.085)	—
MEP Latency (ms)					
Pre-Gel	12.54	(1.35)	12.17	(1.18)	.574
5 min Post-Gel	12.86	(1.14)	12.26	(0.78)	—
15 min Post-Gel	12.95	(1.18)	12.72	(1.20)	—
30 min Post-Gel	12.94	(1.52)	12.62	(0.87)	—
45 min Post-Gel	12.96	(1.62)	12.48	(0.89)	—
Silent Period (ms)					
Pre-Gel	85.67	(19.65)	116.03	(36.36)	.057
5 min Post-Gel	93.20	(28.14)	118.87	(30.85)	—
15 min Post-Gel	83.57	(16.41)	118.10	(37.90)	—
30 min Post-Gel	*96.08	(26.62)	111.50	(41.73)	—
45 min Post-Gel	*94.23	(22.32)	110.47	(41.04)	—
CMEP Area (CMEP/Mmax Ratio)					
Pre-Gel	0.056	(0.039)	0.098	(0.044)	.061
5 min Post-Gel	0.058	(0.042)	0.113	(0.051)	—
15 min Post-Gel	0.069	(0.035)	0.101	(0.037)	—
30 min Post-Gel	0.059	(0.032)	0.100	(0.041)	—
45 min Post-Gel	0.061	(0.042)	0.097	(0.047)	—

CMEP Latency (ms)					
Pre-Gel	7.95	(0.97)	8.93	(2.53)	.325
5 min Post-Gel	7.99	(0.99)	9.00	(2.82)	—
15 min Post-Gel	7.77	(0.84)	8.23	(1.02)	—
30 min Post-Gel	7.84	(0.68)	8.20	(0.88)	—
45 min Post-Gel	7.80	(0.90)	8.14	(1.00)	—
Mmax Area (mV·s)					
Pre-Gel	0.10	(0.04)	0.10	(0.05)	.738
5 min Post-Gel	0.09	(0.04)	0.10	(0.05)	—
15 min Post-Gel	0.09	(0.04)	0.09	(0.04)	—
30 min Post-Gel	0.09	(0.04)	0.10	(0.05)	—
45 min Post-Gel	0.09	(0.05)	0.10	(0.05)	—
Mmax Latency (ms)					
Pre-Gel	4.58	(0.33)	4.77	(0.38)	0.320
5 min Post-Gel	4.75	(0.53)	4.82	(0.81)	—
15 min Post-Gel	6.63	(0.43)	4.67	(0.27)	—
30 min Post-Gel	4.57	(0.46)	4.68	(0.35)	—
45 min Post-Gel	4.65	(0.39)	4.56	(0.28)	—
PPT (kg)					
Pre-DOMS	7.03	(2.48)	5.77	(2.35)	.315
Pre-Gel	3.12	(1.26)	3.51	(1.58)	.370
5 min Post-Gel	3.40	(1.37)	3.66	(2.07)	—
15 min Post-Gel	*3.70	(1.69)	3.69	(1.87)	—
30 min Post-Gel	*3.92	(1.67)	3.77	(2.21)	—
45 min Post-Gel	*4.33	(1.65)	4.12	(2.32)	—

Table 4. Baseline (pre-gel) raw data values for neurophysiological outcome measures in *Experiment A*: No DOMS versus *Experiment B*: DOMS. The *p*-value column indicates between-experiment (No DOMS, *n* = 16; DOMS, *n* = 16) comparisons of baseline outcome measures. *, statistically significant difference, *p* < .05. MEP, motor evoked potential; Mmax, maximal compound motor unit action potential; CMEP, cervicomedullary evoked potential.

Outcome Measure	<i>Experiment A - No DOMS (n = 16)</i>		<i>Experiment B - DOMS (n = 16)</i>		<i>p</i>-value (Pre-)
MEP Area (MEP/Mmax Ratio)	Mean	(SD)	Mean	(SD)	
Pre-Gel	0.222	(0.169)	0.097	(0.057)	*.011
MEP Latency (ms)					
Pre-Gel	11.99	(1.53)	12.36	(1.23)	.468
Silent Period (ms)					
Pre-Gel	77.41	(31.05)	100.85	(32.29)	*.045
CMEP Area (CMEP/Mmax Ratio)					
Pre-Gel	0.186	(0.148)	0.077	(0.045)	*.012
CMEP Latency (ms)					
Pre-Gel	7.46	(1.29)	8.44	(1.92)	.100
Mmax Area (mV·s)					
Pre-Gel	0.073	(0.045)	0.099	(0.041)	.093
Mmax Latency (ms)					
Pre-Gel	4.75	(1.54)	4.67	(0.356)	.844

Figure Legends

Figure 1. Experimental Set-up for (A) *experiment A*: No DOMS and (B) *experiment B*: DOMS.

Figure 2. Comparisons of motor evoked potential (MEP)-related findings across topical analgesic gel (black circles) and placebo gel (white circles) groups ($n = 8$ each), from *Experiment A*: No DOMS (A, C) and *Experiment B*: DOMS (B, D). Panels A and B depict MEP area normalized to maximal compound motor unit action potential (Mmax) area for *Experiments A* and *B*, respectively. Panels C and D show silent period for *Experiments A* and *B*, respectively. Data are expressed as ratios of post-gel time-points (5, 15, 30, 45 min post-gel application) to pre-gel. Circles represent mean and error bars represent the standard error of the mean. *, statistically significant compared to pre-gel value, $p < .05$.

Figure 3. Comparisons of cervicomedullary evoked potential (CMEP)-related findings across topical analgesic gel (black circles) and placebo gel (white circles) groups ($n = 8$ each), from *Experiment A*: No DOMS (A) and *Experiment B*: DOMS (B). Panels A and B indicate CMEP area normalized to maximal compound motor unit action potential (Mmax) area for *Experiments A* and *B*, respectively. Data are expressed as ratios of post-gel time-points (5, 15, 30, 45 min post-gel application) to pre-gel. Circles represent mean and error bars represent the standard error of the mean.

Figure 4. Comparisons of pressure-pain threshold-related findings across topical analgesic gel (black circles/bars) and placebo gel (white circles/bars) groups ($n = 8$ each), from *Experiment B*: DOMS. Data in the larger plot are expressed as ratios of post-gel time-points (5, 15, 30, 45 min post-gel application) to pre-gel. Circles in the larger plot represent mean and error bars represent the standard error of the mean. Data in the inset are expressed as ratios of pre-DOMS to pre-gel. Bars in the inset represent mean and error bars represent the standard error of the mean. *, statistically significant compared to pre-gel value in the topical analgesic gel group, $p < .05$. ‡, statistically significant compared to pre-gel value in the placebo gel group.

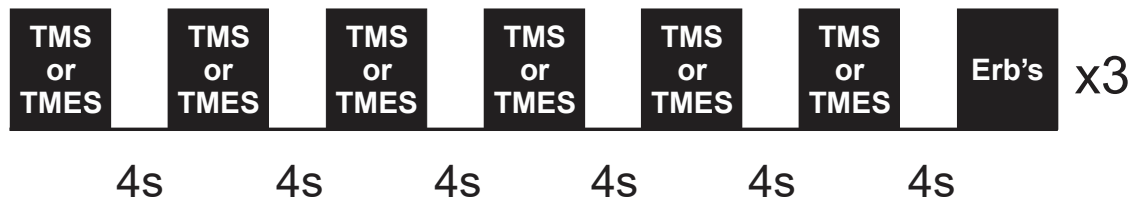
Figure 5. Comparisons of neurophysiological outcomes across *Experiment A*: No DOMS (left) and *Experiment B*: DOMS (right). Panels A and B show ratios of motor evoked potential (MEP) area and cervicomedullary evoked potential (CMEP) area normalized to maximal compound motor unit action potential (Mmax) area, respectively. Panel C shows silent period. Data are expressed as pre-gel values for the entire sample of each experiment ($n = 16$ each). Columns represent the grand mean and error bars represent the standard error of the mean. *, statistically significant compared to *Experiment A*, $p < .05$.

Figure 1.

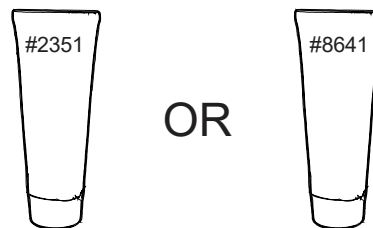
A

No DOMS

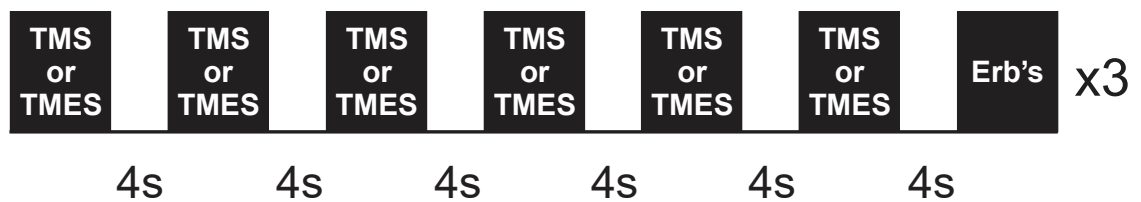
Pre Gel Application



Double Blind Gel Application



5,15,30,45 Minutes Post Gel Application



B

DOMS

Experimental Day 1 - DOMS Protocol

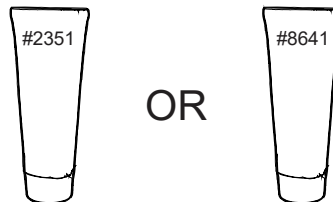


Experimental Day 2 - 48 Hours Post DOMS Protocol

Pre Gel Application



Double Blind Gel Application



5,15,30,45 Minutes Post Gel Application



Figure 2.

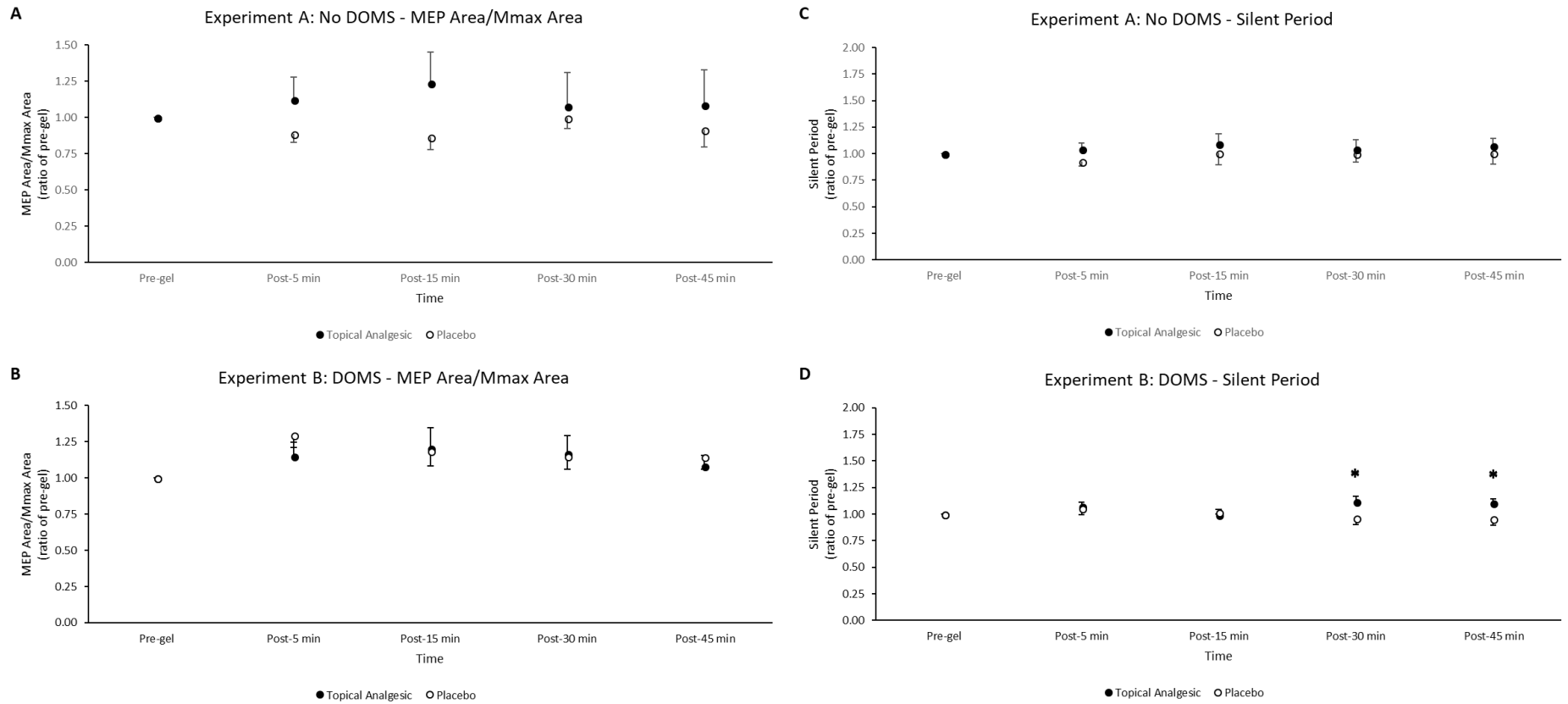


Figure 3.

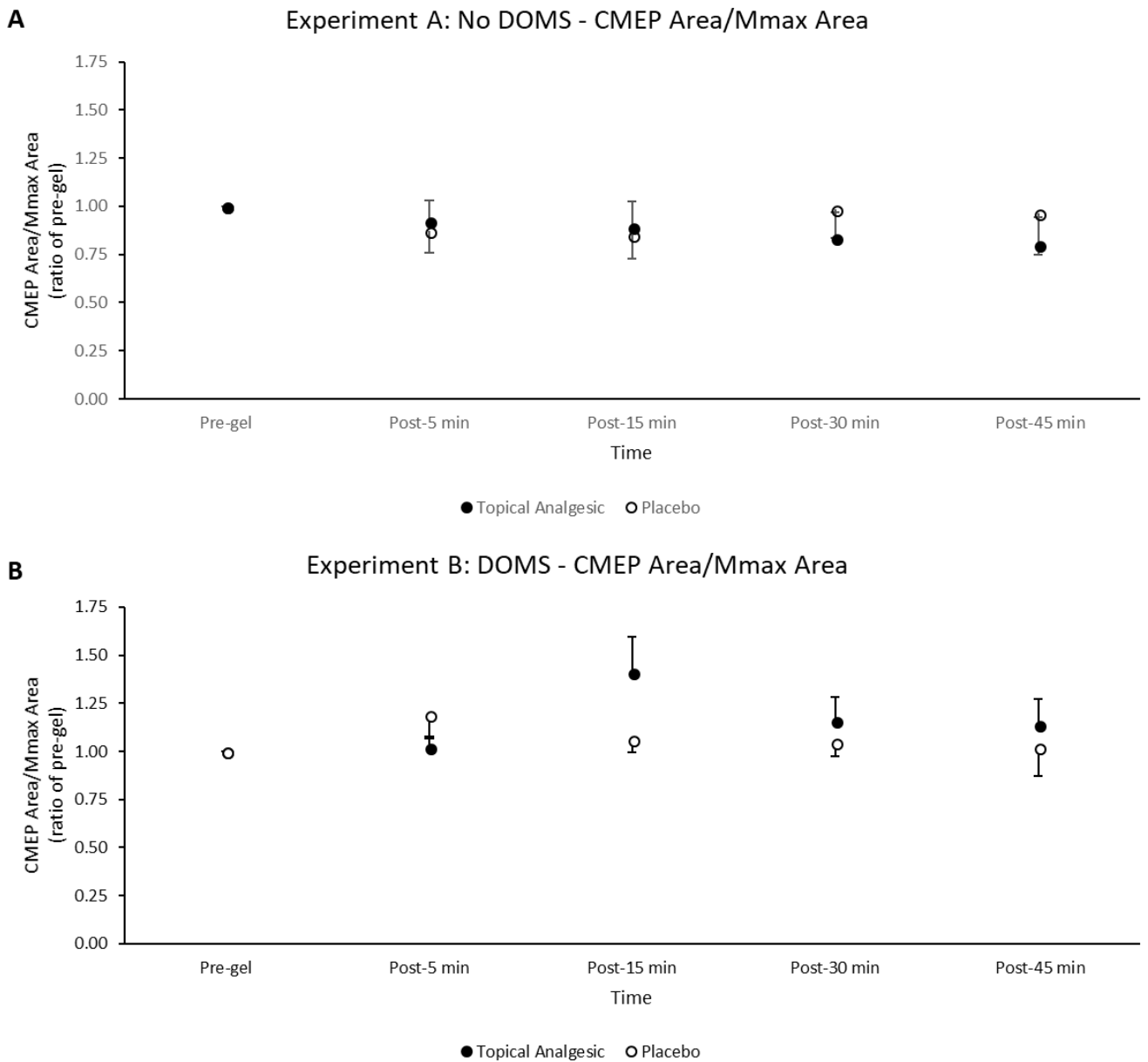


Figure 4.

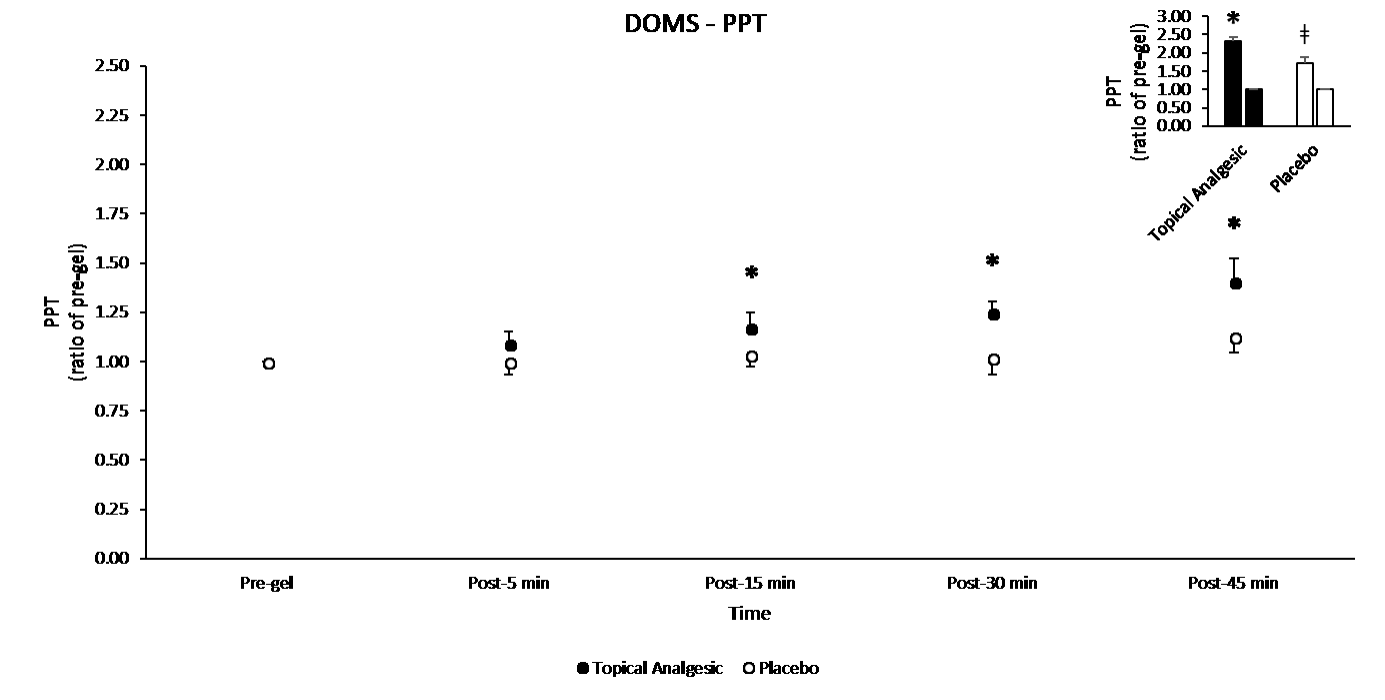


Figure 5.

